IDRC-233e

FINFISH NUTRITION IN ASIA

Methodological Approaches to Research and Development

C.Y. Cho, C.B. Cowey, and T. Watanabe



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IDRC-233e

Finfish nutrition in Asia : methodological approaches to research and development. Ottawa, Ont., IDRC, 1985. 154 p. : ill.

/Fish/, /animal nutrition/, /animal feeding/, /feed supplements/, /research and development/ — /experimentation/, /Asia/, /diet/, /nutritive value/, /fish culture/, /protein rich food/, /vitamins/, /algae/, /conference report/, /list of participants/.

UDC: 597:591.13

ISBN: 0-88936-429-X

Microfiche edition available



ERRATA

Please note the following changes:

Page 19 — lines 35 and 36 should read " . . . with 21% lipid and 35% crude protein in the diet, CP/DE ratio 21 g crude protein/MJ (88 g/Mcal). Japanese workers determined optimal lipid levels to . . . "

Pages 39 and 40 — values in "digestible energy (MJ)" column of "diet formula and composition" table should read 470, 270, 180, 180, 610, —, —, ?, 1710

Page 50 — Fig. 6 should read "Branchial and urinary loss"

Page 54 — Procedure (12) of Table 24 should read
ADC of test ingredient = (ADC of experimental diet − 0.7ADC of reference diet)/0.3

Page 58 — No. 1 of Table 25 should read

$$TC = \frac{\sum(\text{temp. (°C)} \times \text{days})}{Wt^{1/3} - Wo^{1/3}}$$

No. 2 of Table 25 should read

$$Wt(g) = \left[Wo^{1/3} + \frac{\sum(\text{temp. (°C)} \times \text{days})}{TC}\right]^3$$

Page 59 — No. 5 of Table 26, value of 117 kg should be 103 kg throughout

Abstract

Fish nutrition is fundamental to most aquaculture practices in Asia. Although IDRC research support aims, in part, to promote the culture of species requiring no supplementary feeding, it is recognized that formulated feeds are required to increase productivity of many important species now being cultured within the region. This requirement will undoubtedly continue in the future.

Through observing some nutrition research projects, it has become apparent that many of the basic approaches to applied nutrition are not readily available to researchers. Thus, part I of this publication deals with methodological approaches to research and development. Included are discussions on nutrient requirements and deficiencies, fish feeds and their quality, feeding practices, nutrition of broodstock and larvae, and approach and design of nutrition experimentation, as well as an extensive reference and suggested reading list.

Part II presents the proceedings of the Asian finfish nutrition workshop held in Singapore, 23–26 August 1983. Included are research papers dealing with a variety of questions that are important for countries within the region.

Résumé

La nutrition des poissons est une question primordiale pour la plupart des exploitations d'aquiculture en Asie. Le CRDI subventionne des recherches destinées à favoriser la culture d'espèces n'ayant pas besoin d'un supplément alimentaire. Il est cependant conscient que la nourriture commerciale est nécessaire à l'augmentation et au maintien de la productivité de nombre d'espèces cultivées en ce moment dans la région.

L'observation de certains projets de recherche en nutrition a révélé que plusieurs approches fondamentales de la nutrition appliquée ne sont pas facilement accessibles aux chercheurs. La première partie de la publication porte donc sur les approches méthodologiques de la recherche et du développement. Elle aborde notamment les besoins et les carences en matières nutritives des poissons, leur nourriture et sa qualité, les méthodes d'alimentation, la nutrition des géniteurs et des larves, de même que l'optique et la méthode à appliquer à la conception des expériences de nutrition. Elle comprend aussi une importante liste des références et de lectures suggérées.

La deuxième partie, les actes de l'atelier sur la nutrition des poissons qui s'est tenu à Singapour du 23 au 26 août 1983, reproduit des documents de recherche portant sur une gamme de questions importantes pour les pays de la région.

Resumen

La nutrición de los peces es elemento fundamental en la mayoría de las prácticas de acuacultivo en Asia. A pesar de que la ayuda que brinda el CIID a las investigaciones está destinada, en parte, a fomentar la cría de especies que no requieran alimentación complementaria, se admite la necesidad de suministrar piensos formulados para aumentar la productividad de muchas especies importantes que se crian ahora en esta región. Todo parece indicar que esta situación persistirá en el futuro.

El análisis de algunos proyectos de investigaciones sobre nutrición ha revelado que los investigadores no tienen fácil acceso a muchos enfoques básicos en el campo de la nutrición aplicada. Es por esto que en la primera parte de esta publicación se analizan enfoques metodológicos relativos a las actividades de investigación y desarrollo. Los temas tratados en la primera parte incluyen: necesidad y carencia de nutrientes; piensos para peces y la calidad de los mismos; prácticas de alimentación; nutrición de las crías y larvas; y principios y diseño de experimentos sobre nutrición. También se incluye una extensa lista de referencias y de lecturas complementarias.

La segunda parte recoge los debates del seminario sobre nutrición de los peces en Asia, celebrado en Singapur del 23 al 26 de agosto de 1983. Se incluyen algunas ponencias que tratan de muchas cuestiones de importancia para los países de la región.

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Foreword

The International Development Research Centre (IDRC) of Canada has been providing support for research on aquaculture since the initiation of its fisheries program in Asia in 1973. The mechanism of holding small informal workshops with researchers who work specifically in this field has proven useful for allowing improved regional researcher contact and exchange of information. It also has the advantage of assisting IDRC to establish guidelines for future research funding.

Fish nutrition is fundamental to most aquaculture practices in Asia. Although IDRC research support aims, in part, to promote the culture of species requiring no supplementary feeding, it is recognized that formulated feeds are required to increase productivity of many important species now being cultured within the region. This requirement will undoubtedly continue in the future.

In planning for this workshop, it became apparent that little detailed nutritional information is presently available on Asian tropical finfish. The resource persons' presentations, therefore, have drawn very heavily upon examples from cold-water species in North America, Europe, and Japan.

A number of the workshop participants are already carrying out nutrition research as part of IDRC-supported projects. Through observing these projects, it became apparent that many of the basic approaches to applied nutrition were not readily available to the researchers. It was decided, therefore, that part of the workshop should be devoted to methodological approaches, which were the basis of the resource persons' presentations.

Knowledge on feed ingredients and finfish feeds has developed on an empirical basis in Asia but only a few nations have any sort of commercial production of fish feed. It was encouraging, therefore, that several commercial feed manufacturers participated in this meeting as a stronger regional fish-feed industry must develop if increased fish production through intensification is going to take place. An improved understanding of the nutrition and feeding behaviour of the major cultivable species in the region and a stronger working relationship between the fish farmer, feed industry, and researchers is desirable. Hopefully, this forum assisted in improving such dialogue.

IDRC wishes to thank all those who participated in the workshop, particularly Drs C.Y. Cho, C.B. Cowey, and T. Watanabe who gave very generously of their time and extensive knowledge of finfish nutrition. The workshop also benefited greatly from the presence of Jan Hildingstam and his contributions from the point of view of the commercial feed manufacturer. Finally, appreciation is extended to the Aquaculture Unit, Primary Production Department, Changi, Singapore, for arranging a field visit to its research sites.

IDRC hopes to continue to provide research support in the field of fish nutrition in the future and that a network of related projects in Asia will be developed. A proposal was made to convene similar meetings in the future, thereby continuing the process of information exchange.

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Future Plans and Recommendations

- It was agreed by all participants that a large amount of research remains to be carried out in Asia in the field of fish nutrition. Due in part to differing levels of experience and training of researchers in the region, the variety of species cultured, and their related diet requirements, it proved very difficult to arrive at region-wide recommendations.
- Most countries felt that a higher level of applied training for researchers and improved research facilities were needed. Although most countries have established partial nutrition laboratories and tank facilities, these may not allow for detailed testing of the developmental stages of fish under the various categories of nutritional requirements.
- Some countries placed greater emphasis on the establishment of detailed nutritional requirements for selected stages of development, such as larval diets, whereas others preferred to emphasize the initial development of practical production diets that could be immediately used by fish farmers. However, it was emphasized that production (grower) diets represent more than 80% of total feed requirements and have the greatest impact on the socioeconomic aspects of aquaculture in the region.
- Many countries have a major program for artificial spawning of important species. Research on the development of suitable broodstock and larvae diets, therefore, was planned despite notes of caution on the difficulties involved in successfully developing such diets.
- All countries emphasized the need, or at least desirability, of using local ingredients where possible. Digestibility studies on primary feed ingredients and diets, working toward "least-cost formulations," are planned. It was recognized that researchers will have to conduct comparative economic studies during this process as the same ingredients are used by other animal-feed industries. A close working relationship with the feed industry, therefore, was strongly urged.
- It was felt that more emphasis should be placed on research on the relationship between essential nutrients and energy, particularly with respect to feeding larvae and fry. This research should be combined with continued studies on essential amino acid and fatty acid requirements. Vitamins and minerals, although important, should receive less attention until adequate research has been carried out on some of the above nutrients.
- Despite differences in opinion on future research plans, it was agreed that meetings of this type are very useful. Increased information exchange among researchers in the region is planned and a directory of active fish nutrition researchers will be developed.

Part I

Methodological Approaches to Research and Development

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Introduction

In culturing fish in captivity, nothing is more important than well-balanced diets and adequate feeding. If there is no utilizable feed intake by the fish, then there will be no growth and death eventually results. An undernourished or malnourished fish is never able to maintain its health and be productive, regardless of the quality of its environment.

The production of nutritionally balanced diets for fish requires research, quality control, and biological evaluation. Figure 1 outlines the importance of the various groups needed to develop a successful diet and feeding program.

Malnutrition obviously impairs fish productivity and results in deterioration of health until recognizable diseases ensue. The borderline between reduced growth and diminished health, on the one hand, and overt disease, on the other, is always very difficult to define. Therefore, the ability to recognize a deterioration in performance during its initial stages and take

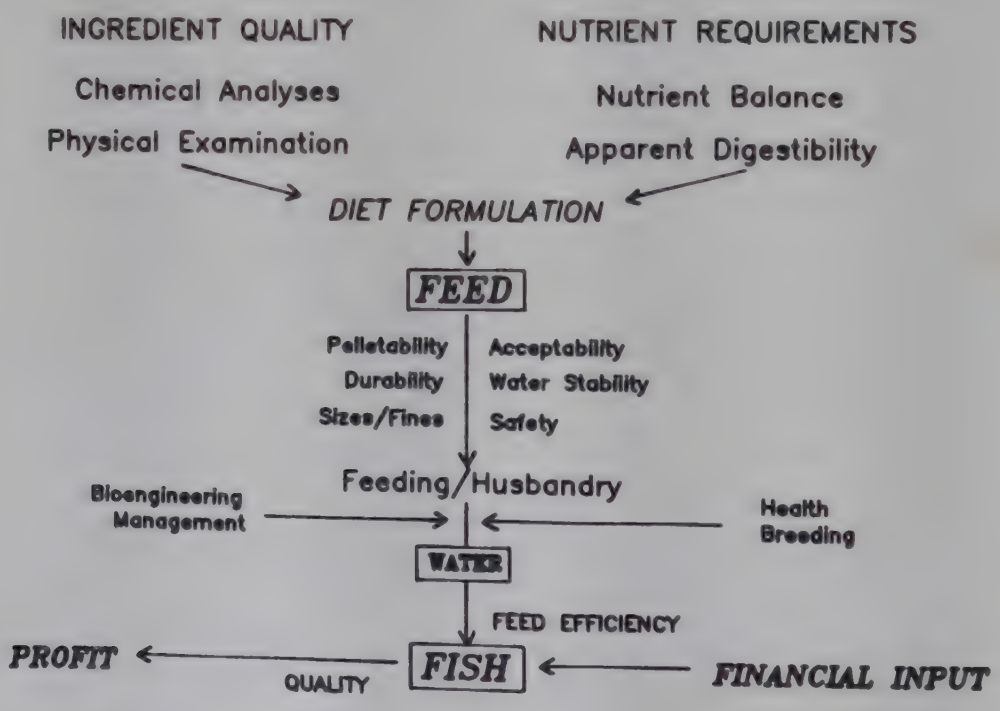


Fig. 1. Fish nutrition and aquaculture.

corrective action will remain an essential part of the skill of the fish culturist.

Diets can negatively influence the well-being of a fish by inducing nutrient deficiencies, imbalances, or toxicoses or by introducing infective agents into the fish. A well-balanced diet, however, not only results in higher production but also provides the nutrients necessary to hasten recovery from diseases or aid the fish in overcoming the effects of environmental stress. In some cases, a good-quality diet may slow the progress of idiopathic diseases. Hence, nutritionally balanced and quality-controlled diets for fish production are of critical importance. Therefore, before attempting to culture fish, it would be wise to ask the fundamental question "What and how should I feed my fish?"

Current Status of Finfish Nutrition: Nutrient Requirements and Deficiencies

Protein and Amino Acids

For most animals, the protein requirements are considered to be the sum of the requirements for individual essential amino acids and the requirements for nonessential nitrogen. Although attempts have been made with many species of fish to establish an optimal dietary protein level, this information alone, without recourse to data on essential amino acid requirements, is of limited value and may mask a number of factors.

Protein Requirements

General protein requirements for a number of species are shown in Table 1. The values were obtained from dose–response (weight gain) data in which the diets used were intended to be isoenergetic. This intention was not always realized, however, as, on occasion, starch or dextrin was substituted for protein on a weight basis in low-protein diets. The inherent assumption that protein and complex carbohydrates have similar metabolizable energy values for fish, therefore, is not valid.

Table 1. General protein requirements of certain fish species.

Species	g protein/kg dry diet	Reference
Rainbow trout (*Salmo gairdneri*)	400–460	Satia (1974); Zeitoun et al. (1976)
Common carp (*Cyprinus carpio*)	310–380	Ogino and Saito (1970); Takeuchi et al. (1979)
Channel catfish (*Ictalurus punctatus*)	220–400	Garling and Wilson (1976)
Tilapia (*Tilapia zillii*)	350	Mazid et al. (1979)
Japanese eel (*Anguillo japonica*)	445	Nose and Arai (1972)

The values shown in Table 1 are, in general, high. This is to be expected of carnivorous species but it is surprising for those that are omnivorous or even herbivorous. Thus, the possibility that the method employed to obtain the data tends to result in high values cannot be discounted. At high dietary protein levels, therefore, assimilated amino acids may give rise to intracellular concentrations greater than those that can be used rapidly for protein synthesis and high rates of amino acid oxidation may result. Because the utilizable energy available to the fish from dietary protein almost certainly exceeds that obtainable from carbohydrates, better growth may result. This view is echoed in the suggestion of Jackson and Capper (1983), from work on tilapia, that "energy provided by protein catabolism leads to improved growth at high protein levels."

Biotic and Abiotic Factors

Dietary protein requirements are said to be size dependent, i.e., smaller fish require higher levels of protein for maximal growth than larger fish. Data illustrating this for channel catfish are presented in Table 2. The same is true of rainbow trout and coho salmon.

Experiments carried out on chinook salmon about 25 years ago were interpreted as showing an increase in dietary protein requirement with temperature (DeLong et al. 1958). Analysis of the dose–response curves involved, however, revealed that they were inadequate, with no plateauing of the response curve being evident at high protein intake in some of the experiments. Similarly, Possompes (1973), using a dietary protein range of 21–60% in experiments on rainbow trout, found a linear increase in weight gain with an increasing protein inclusion rate at 10 and 17 C — again with no leveling off in response. Many physiological functions accelerate with an increase in environmental temperature, e.g., food intake is enhanced as is the gastrointestinal transit rate, but the efficiency with which both protein and energy are digested by rainbow trout did not alter with a change in temperature within the normal temperature range. It is possible, therefore, that differential utilization of protein and energy is taking place. This may indicate that dietary protein/energy ratios should be altered to meet changes in environmental temperature, particularly as fish appear to be well adapted to dealing with temperature changes within their thermal tolerance limits.

Table 2. Protein requirements of channel catfish of different size.

Dietary protein (g/kg)	Digestible energy (kJ/kg)	Small fish		Large fish	
		Initial weight (g)	Final weight (g)	Initial weight (g)	Final weight (g)
250	9.7	14	97	114	526
350	10.7	14	126	114	497

Source: Wilson and Robinson (1982).

Table 3. Essential amino acid requirements (g/kg dry diet) at stated dietary protein levels of certain fish.

	Chinook salmon	Japanese eel	Common carp	Channel catfish
Arginine	24	17	16	10.3–17.0
Histidine	7	8	8	3.7
Isoleucine	9	15	9	6.2
Leucine	16	20	13	8.4
Lysine	20	20	22	15.0
Methionine + cystine	16	19	12[a]	5.6[a]
Phenylalanine + tyrosine	21	22	25	12.0
Threonine	9	15	15	5.3
Tryptophan	2	4	3	1.2
Valine	13	15	14	7.1
Protein in diet	400	377	385	240.0

Source: NRC (1983).
[a] In the absence of cystine.

Quantitative Amino Acid Requirements

Traditionally, quantitative amino acid requirements of terrestrial animals have been measured using dose–response curves, the response measured being weight gain. Although alternative methods, such as those based on the plasma concentrations of free amino acids, have been applied in recent years, recommendations have usually been based on weight-gain measurements. For certain amino acids, plasma or serum concentration and whole body oxidation rates have proven to be a useful adjunct to weight-gain measurements.

The values obtained using these methods for a number of species are presented in Table 3. Some differences between species are apparent; whether or not these are real, however, remains to be confirmed. An additional degree of confidence in the results may emerge if the same or similar values are obtained by different laboratories for one species.

In measuring nutrient requirements, rapid growth rates that are comparable (for the complete diet) with those obtained under practical conditions are desirable. This will depend on the acceptability of the food and the feeding regime adopted. Practices vary from the use of a restricted ration, usually defined in terms of percentage of body weight per day, given at several meals, to feeding fish to satiation several times per day. It is clear that different feeding practices have led to different levels of food intake in different laboratories. Because of this, or because accurate measurement of food intake may sometimes be difficult (especially with very small fish), nutrient requirement values have usually been expressed as a percentage of dietary protein or as a percentage of the diet (Table 3).

Data expressed as dietary concentrations, however, do not provide information about the efficiency of amino acid utilization. A low value for a given amino acid in one species compared with another may mean that either they utilize that amino acid more efficiently or they consume more food. The requirements of channel catfish and rainbow trout for certain amino acids appear very different when expressed as dietary concentrations but it can be seen from Table 4 that the intake of these amino acids to sustain maximal rates of growth is quite similar. Thus, differences in

Table 4. Amino acid requirements, expressed as percentage of diet and percentage of body weight per day, of trout and channel catfish.

Amino acid	Percentage of diet		mg/100 g body weight·day^{-1}	
	Trout	Catfish	Trout	Catfish
Arginine	1.80	1.03	28	31
Lysine	2.18	1.50	43	45
Methionine	1.00	0.56	20	20
Tryptophan	0.24	0.12	4.7	3.6

requirements, when expressed as dietary concentrations, should not necessarily be accepted at their face value.

When carrying out dose- response experiments on amino acids, it is frequently necessary, to enable a graded dose to be given, for the "protein" component of the diet to consist of both free amino acids and protein. It has been shown repeatedly, in several species, that the weight gain of fish given a diet composed entirely of protein is greater, often markedly so, than that obtained with a diet identical in overall amino acid composition but composed of both free amino acids and protein. Data illustrating this point for channel catfish and rainbow trout are presented in Table 5. Thus, the maximal response indicated from dose- response (growth) curves may be well below the maximum growth attainable under those conditions. This will militate against the accuracy and reliability of the values obtained.

Utilization of Free (Nonprotein) Amino Acids

The efficiency with which dietary free amino acids are used by fish is a matter of controversy. Although Wilson et al. (1977) were able to use diets containing large amounts of free amino acids (even though growth was inferior to that obtained with protein in the diet), Andrews and Page (1974) were not able to improve the growth of channel catfish by supplementing diets limited in sulphur amino acids with methionine or cystine.

Subsequently, Andrews et al. (1977) were not able to increase the growth rate of channel catfish given an arginine-deficient diet through the addition of arginine-HCl. The addition of protein-bound arginine to the diet, however, lead to an increase in growth rate. Experiments of this type have led to the view that channel catfish cannot efficiently utilize free amino acids added to diets that are relatively deficient in one or more amino acids. This view may need to be reconsidered, however, due to the fact that Robinson et al. (1980) obtained enhanced growth when lysine-deficient diets containing peanut meal were supplemented with lysine, although the

Table 5. Growth of channel catfish and trout given diets in which the protein component was composed either entirely of protein, as such, or as a mixture of free amino acids and protein.

	Percentage increase in initial body weight	
	Channel catfish	Trout
24% whole-egg protein	319	—
5.7% protein + 22% free amino acids	189	—
50% casein	—	312
25% casein + free amino acids	—	204

enhanced growth was substantially less than that obtainable using whole-egg protein.

The answer may lie in the rate at which amino acids (supplied either free or as components of protein) reach sites of protein synthesis. The nature of protein synthesis requires that all amino acids be available within the cell at the same time. Presumably, this need is met in catfish when large amounts of amino acid mixtures are used in the diet, but is not always met when deficient proteins are supplemented singly with amino acids. However, supplementation of a single deficient amino acid to some proteins is a practical tool in spite of its potentially less efficient utilization.

The frequency of feeding and meal size will affect this issue. A single large meal is less likely to be effective when supplementation of protein with free amino acids is being attempted than many small meals distributed at equal time intervals. Yamada et al. (1981) found this to be the case when they obtained good growth rates by feeding a diet containing large amounts of free amino acids to fry at 18 feedings (10% body weight) over 24 hours but much lower rates when the ration was given at only three feedings.

It is clear that doubts remain about apparent differences in amino acid requirements of different species. These stem mainly from interpretation of the dose–response curves, the growth rates obtained when large amounts of free amino acids are used in diets, and the lack of supporting data from parameters other than weight gain.

Essential Amino Acid Balance in the Diet

At the level of amino acid deposition in tissues, the composition of the product being formed, largely muscle, should provide a useful guide to the pattern of amino acids required. In fact, among monogastric farm animals it has been suggested that this pattern may closely relate to requirements in the diet. In young fish, the product being formed is predominantly muscle and, as fish do not appear to have any large maintenance requirements for protein, the ratio of amino acids in muscle protein may represent a pattern best suited to the nutritional needs of the fish.

In Table 6, the ratio of essential amino acids in fish muscle is compared with the requirement pattern found in feeding experiments carried out on four species of fish. Because the overall amino acid composition of muscle protein does not differ greatly between species, it had been hoped that there might be good correlation between requirement and muscle patterns. The agreement between ratios for some essential amino acids (valine, histidine) is good but it is variable for other amino acids. A detailed comparison of over- and underestimates, however, cannot be justified at this stage. Thus, there is some way to go before a general consensus is reached on an optimal essential amino acid pattern for fish diets.

Lipids, Fatty Acids, and Energy

Dietary lipids serve both as sources of essential fatty acids (EFA) and energy. In addition, they also act as carriers of fat-soluble vitamins. Recent studies of EFA in fish suggest that requirements may differ from species to species.

Table 6. Relative proportion of essential amino acids in fish muscle (weight of each essential amino acid as a percentage of the total weight of all essential amino acids) together with recommended requirements of four species of fish, also expressed as a percentage by weight of the requirement for all essential amino acids.[a]

	Fish muscle[b]	Chinook salmon	Channel catfish	Japanese eel	Common carp
Arginine	11.16	17.5	13.8	11.5	11.7
Cystine/2	2.8	—	—	—	—
Histidine	5.79	5.1	4.9	5.4	5.8
Isoleucine	8.06	6.6	8.3	10.1	6.6
Leucine	14.80	11.7	11.2	13.5	9.5
Lysine	16.97	14.6	20.1	13.5	16.1
Methionine	5.32	11.7	7.5	8.1	8.8
Phenylalanine	7.58	15.3	16.0	14.9	18.3
Threonine	8.85	11.7	7.1	10.1	10.9
Tryptophan	2.29	1.5	0.9	2.7	2.2
Tyrosine	7.57	—	—	—	—
Valine	9.34	9.5	9.5	10.1	10.2

[a] Refer also to Cowey and Tacon (1983).
[b] Connell and Howgate (1959).

Essential Fatty Acids

Many of the early studies of EFA requirements were performed on salmonids, and especially on rainbow trout. These were shown, through the use of defined diets, either free of fat or containing only known fatty acids of particular configurations, to require fatty acids of the linolenic (n-3) series. (The designation (n-3) refers to the position of the first double bond in the molecule relative to the methyl terminal; in this instance, it occurs three carbon atoms from the methyl carbon; successive double bonds are methylene interrupted.) In the absence of such fatty acids, the fish suffered certain pathologies, had poor growth rates, and low rates of food conversion.

Castell et al. (1972a,b) showed that the requirement of rainbow trout for linolenic acid (18:3(n-3)) is 1% of the diet and no combination of 18:3(n-3) with linoleic acid 18:2(n-6) resulted in as fast a growth rate or as efficient a feed conversion ratio as did 1% 18:3(n-3) alone in the diet. Inclusion of 18:2(n-6) in the diet resulted in some improvement in growth and feed conversion compared with an EFA deficient diet but (n-6) series fatty acids did not prevent some EFA deficiency symptoms from appearing. Later experiments conducted by Watanabe (1982) placed the linolenic acid requirement of rainbow trout at between 0.8 and 1.6% of the diet. Fish given diets containing less than 0.5% 18:3(n-3) had retarded growth, erosion of the caudal fin, and a shock syndrome caused by physical irritation of the fish.

The effect of total dietary lipid level on EFA (linolenic acid) requirements of rainbow trout was also examined by Watanabe and colleagues. They showed that as the level of lipids in the diet increased so did the requirement for linolenic acid. They suggested that EFA requirements be expressed as a proportion of dietary lipid, with the requirement of rainbow trout for linolenic acid being postulated as 20% of the dietary lipids. This fact is especially important in practical diets for rainbow trout, which require a relatively large amount of lipid as a dietary energy source.

The EFA requirements of two of the most heavily studied warm-water fish, namely channel catfish (*Ictalurus punctatus*) and carp (*Cyprinus carpio*), appear to be far less than those of rainbow trout. When channel catfish were given diets containing beef tallow, olive oil, or menhaden oil, for example, high weight gains were recorded. When sunflower oil (high in 18:2 (n-6)) or linseed oil (high in 18:3(n-3)) were used as dietary lipid, on the other hand, lower weight gains were obtained. In addition, catfish given diets free of fat revealed no EFA deficiency symptoms apart from a reduction in growth rate. Similarly, Watanabe and his colleagues were able to grow carp fingerlings of about 2.5 g initial weight on a diet lacking fat for a fairly long period without any appreciable problems.

Adding saturated fat to the diet elicited a positive growth response but supplements of 18:3(n-3) and 18:2(n-6) to the diet for a period of 22 weeks resulted in little further improvement in growth and feed conversion. The relatively low growth rate of carp given the fat-free diet was attributed to its lower energy content because of the absence of fat. When experiments were carried out with very small carp weighing about 0.65 g, which had been kept on a fat-free diet for 4 months before starting the feeding trial, it was clearly shown that these fish had an EFA requirement for both 18:2(n-6) and 18:3(n-3). The best growth and feed conversion rates were obtained in carp given a diet containing both 1% 18:2(n-6) and 1% 18:3(n-3).

Eel (*Anguilla japonica*), another important warm-water fish, also requires dietary sources of both 18:2(n-6) and 18:3(n-3) for optimal performance, 0.5% of each acid being necessary. The tropical herbivorous *Tilapia zillii*, which may live in either freshwater or seawater, grows better when 18:2(n-6) or 20:4(n-6) are included in the diet than when 18:3(n-3) or 20:5(n-3) are included, indicating a requirement for (n-6) rather than (n-3) series fatty acids. The requirement of *Tilapia* is about 1% 18:2(n-6), 1% 20:4(n-6), or a combination of the two totaling 1% in the diet.

The polyunsaturated fatty acids esterified to the 2 position of the phosphatide in biomembranes are generally long-chain acids of 20 or 22 carbon atoms. Thus, when an EFA of 18 carbon atoms is supplied in the diet this is normally chain elongated and further desaturated to a 20- or 22-carbon acid suitable for incorporation in the phosphatide. Conversely, if a 20- or 22-carbon EFA is supplied (at least in the diet of some omnivorous mammals), it has greater EFA activity than the 18-carbon precursor.

In 1975, it was clearly demonstrated that turbot (*Scophthalmus maximus*) are incapable of converting (by chain elongation and further desaturation) 18-carbon EFA (18:3(n-3) or 19:2(n-6)) to the corresponding longer chain acids (22:6(n-3) or 20:4(n-6)) at rates concomitant with normal growth and health. Consequently, the longer chain, more highly unsaturated fatty acids (in this instance of the (n-3) series, i.e., 22:6(n-3)) must be supplied preformed in the diet.

It soon became clear that several other species are not capable of desaturating and chain elongating 18-carbon fatty acids; most of these require (n-3) series fatty acids and for them linolenic acid (18:3(n-3)) is of little value. These species include red sea bream (*Pagrus major*), black sea bream (*Mylio macrocephalus*), opal eye (*Girella migricans*), and yellow tail (*Seriola quinqueradiata*). Red sea bream and yellow tail are both commercially important cultured warm-water marine fish in Japan. For these

species, a fish oil containing highly unsaturated fatty acids must be supplied preformed in the diet.

Lipids as a Dietary Energy Source

Lipids play an important role as an energy source in fish diets, especially for carnivorous fish in which the availability of dietary carbohydrates for energy is low. The gross energy of lipids (heat of combustion) amounts to 39.4 kJ/g (9.45 kcal), but physiological values (i.e., making allowance for digestibility) amount to 33.5 kJ (8 kcal) for saturated and 37.7 kJ (9 kcal) for unsaturated fats respectively.

A digestible energy basis presently seems to be the most sensible way of dealing with the energy value of ingredients even though metabolizable energy values of a defined diet are, theoretically, a more exact measure of the dietary energy utilizable for metabolism by the tissues of the animal. Direct measurement of metabolizable energy in fish, however, is difficult and involves confining the fish in small volumes of water in metabolism chambers. Indirect estimation of metabolizable energy values by Phillips and Brockway (1959) ("modified" Atwater's (1899) physiological fuel values) has not led to any considerable measure of agreement among different groups of workers. In particular, experimentally determined values for heat increment vary widely and their magnitude in fish is a controversial issue. Nevertheless, in deriving an energy budget, a generally acceptable value for heat increment must be included.

In common with other animals, many species of fish are known to eat to meet their energy requirements. Given that the food has a satisfactory nutrient balance, fish are then, within limits, able to compensate for a low energy density in the food by eating more of it. This type of compensation will occur up to the limits of the physical capacity of the digestive tract, although fish given a high energy density diet will require less feed per unit of weight gained. At maximal physical intake, however, fish given a high energy density diet can ingest more nutrient and will, of course, sustain a higher growth rate.

Provision of an optimal balance of energy components in the diet is important because an excess or deficiency of nonprotein energy (lipid and carbohydrate) may result in lowering the growth rate. If the diet is deficient in nonprotein energy, protein will be used for energetic purposes (basal metabolism and voluntary activity) rather than for protein synthesis. Similarly, if the diet contains an excess of nonprotein energy, appetite or demand may be satisfied before a sufficient quantity of protein (and possibly other nutrient) is ingested to satisfy demand for maximal rates of protein synthesis and growth.

It may be argued that an optimal balance of energy components is difficult to define. For many species, dietary protein levels can be reduced and dietary lipid levels increased from a given "control" point without lowering the food conversion rate or growth rate. However, this is accomplished at the expense of an increasingly fatty carcass. For example, when rainbow trout of initial body composition 6.3% lipid, 16.7% protein, and 75.1% water were given diets containing 24% herring oil and 35% protein, they had, 18 weeks later, a body composition of 12.9% lipid, 15.3% protein, and 70.3% water. When the diet contained 8% herring oil and 53% protein, body composition was 9.4% lipid, 16.6% protein, and

72.3% water after 18 weeks. There was no difference in the mean weight gain or food conversion ratio between these treatments.

Changes in body composition of the farmed fish from that of its counterpart in the wild may have consequences for marketing. These may be more important for some species than others. A small (2–3%) increase in the lipid content in the muscle of species that store fat in their muscles (such as salmonids) seems unlikely to affect market acceptability. However, for a species (such as turbot) that does not store fat in its muscle (content well below 1% on a fresh-weight basis), an increase of 2.3% in muscle lipid could be seen as altering the quality of the product considerably.

Another factor to be considered is the intended fate of the fish being cultivated. If it is to be used directly as food, the use of high lipid diets leading to the deposition of large amounts of visceral and muscular fat is undesirable. If, on the other hand, the fish is being reared for release into the natural environment, the laying down of substantial reserves of fat is a sound strategy.

For those species that have a wide thermal tolerance, both metabolic rate and feed intake will increase with an increase in water temperature. In these species, high energy rations are likely to be important for supporting growth at high temperatures.

Many experiments have been carried out on channel catfish and rainbow trout to determine the level of dietary lipid that affords the maximal protein-sparing effect. Consequently, dietary energy requirements have often been expressed as a function of dietary protein level. For channel catfish of 14 g live weight grown at a temperature of 27°C, the best growth was obtained with diets containing 35% crude protein and 12% lipid, whereas fish larger than 100 g grew well with 25% crude protein and 12% lipid in the diet. Overall, the optimal crude protein : digestible energy (CP/DE) ratio for growth was 25 g crude protein/MJ (103 g/Mcal).

For rainbow trout, factorial experiments (in which weight gain, feed conversion, and protein utilization were measured) using several lipid levels at each of a number of different dietary protein levels and carried out by different laboratories have produced similar results. In an experiment carried out at 12°C, American workers obtained maximal protein sparing with 21% lipid and 35% crude protein in the diet, CP/DE ratio 21.4 g crude protein/MJ (88 g/Mcal). Japanese workers determined optimal lipid levels to be somewhat lower at 15–20%, again with 35% crude protein. It has similarly been found possible to reduce the protein level in diets for marine fish, in the case of yellow tail from 70 to 55%, without retardation of growth, if the energy content is maintained at a high level with pollock-liver oil. In contrast with these results, effects seem to be less marked in omnivorous fish, such as carp, which can more effectively use carbohydrate, in addition to lipid, as a dietary energy source.

In the past, utilization of lipids as an energy source in diets for fish under cultivation has frequently involved using oil containing a high degree of unsaturated fatty acids. Perhaps this is because most fish lipids are relatively unsaturated and it would be desirable, therefore, to avoid altering the characteristics of the fish being harvested. It was shown many years ago, however, that when channel catfish were fed a diet containing 10% beef tallow (41% of the fatty acids being saturated) for 10 weeks at 20°C, the fatty acid content of the carcass, in terms of saturated fatty acids, did not differ

from that of catfish given diets containing 10% menhaden oil (25% of the component fatty acids saturated). This demonstrated that it is possible to supply at least a portion of the dietary lipid in a saturated form.

It has since been shown that saturated fat can also be used in moderation, and with impunity, in diets for rainbow trout. For example, there were few changes in the composition of total body lipid in rainbow trout when half of the lipid (22% herring oil) was substituted with animal fat (lard) containing a high proportion of saturated fatty acids.

Vitamins

Vitamins are complex organic substances, usually of comparatively small molecular size (molecular weight usually less than 1000). They are distributed in feedstuffs in small quantities and form a distinct entity from other major and minor food components. Vitamins are needed for normal growth, maintenance, and reproduction of animals. The absence of any vitamin from the diet was formerly regarded as leading to specific deficiency diseases in mammals and birds. In fish, many of the vitamin-deficiency symptoms are nonspecific (Table 7).

So far, four fat-soluble and 11 water-soluble vitamins are known to be required by fish. Many of the water-soluble vitamins function either directly or in a modified form as a coenzyme for one or more enzymes, e.g., pyridoxal phosphate serves as a coenzyme for virtually all amino-transferases, thiamine as a coenzyme for co-carboxylase, and riboflavin as a coenzyme for glutathione reductase and D-amino acid oxidase. In certain instances, this functional role of vitamins has been used to advantage as a means of assessing the nutritional status of an animal with respect to that vitamin. The enzyme activity in a physiological extract of a tissue may be assayed, the tissue extract then incubated with the modified vitamin (coenzyme), and the activity re-assayed. The ratio of the first activity value to the second may provide an index of the extent to which the diet is fulfilling the requirement for the vitamin in question. To be valid, this approach requires careful establishment of the causal relationship between maximal enzyme activity and other basic parameters such as growth rate and freedom from pathology.

None of the fat-soluble vitamins is known to function as a coenzyme. Vitamin A is involved in the metabolism of mucopolysaccharides and visual pigments and for the general maintenance of epithelial tissues. Vitamin D functions in calcium homeostasis, possibly by induction of calcium-binding proteins. Vitamin E is a lipid-soluble antioxidant and may terminate peroxidative chain reactions among the highly unsaturated fatty acids of biomembranes. Finally, vitamin K is involved in electron transport and oxidative phosphorylation; it is also a cofactor in the blood coagulation process.

The requirement for a vitamin may be affected by the composition of the diet, e.g., the requirement for vitamin E may increase as the polyunsaturated fatty acid level in the diet increases. Watanabe and his colleagues demonstrated that elevated levels of linolenic acid in carp diets led to an increased requirement for vitamin E. Similar results have been obtained with rainbow trout given diets containing varying levels of fish-liver oils. Recommended levels of vitamins in salmonid diets are summarized in Table 8.

Table 7. Nutritional deficiency symptoms in finfish.

Code	Symptom	Possible nutrient deficiencies
01	Anemia	Folic acid, inositol, niacin, pyridoxine, riboflavin, rancid fat, vitamins B_{12}, C, E, and K
02	Anorexia (poor appetite)	Biotin, folic acid, inositol, niacin, pantothenic acid, pyridoxine, riboflavin, thiamine, vitamins A, B_{12}, and C
03	Ascites	Vitamins A, C, and E
04	Ataxia	Pyridoxine, pantothenic acid, riboflavin
05	Atrophy: Gills	Pantothenic acid
06	Muscle	Biotin, thiamine
07	Calcinosis, renal	Magnesium
08	Cartilage abnormality	Vitamin C, tryptophan
09	Cataract	Methionine, riboflavin, thiamine, zinc
10	Ceroid liver	Rancid fat, vitamin E
11	Cloudy lens	Methionine, riboflavin, zinc
12	Clubbed gills	Pantothenic acid
13	Clotting blood, slow	Vitamin K
14	Colouration, dark skin	Biotin, folic acid, pyridoxine, riboflavin
15	Convulsions	Biotin, pyridoxine, thiamine
16	Discolouration of skin	Fatty acids, thiamine
17	Deformation: Bone	Phosphorus
18	Lens	Vitamin A
19	Degeneration of gills	Biotin
20	Dermatitis	Pantothenic acid
21	Diathesis, exudative	Selenium
22	Disease resistance, low	Protein, vitamin C
23	Distended stomach	Inositol
24	Dystrophy, muscular	Selenium, vitamin E
25	Edema	Niacin, pyridoxine, thiamine, vitamins A and E
26	Epicarditis	Vitamin E
27	Equilibrium loss	Pyridoxine, thiamine
28	Erosion of fin	Fatty acids, riboflavin, vitamin A, zinc
29	Exophthalmos	Pyridoxine, vitamins A, C, and E
30	Exudated gills	Pantothenic acid
31	Fatty liver	Biotin, choline, fatty acids, inositol, vitamin E
32	Feed efficiency, poor	Biotin, calcium, choline, energy, fat, folic acid, inositol, niacin, protein, riboflavin
33	Fragility: Erythrocytes	Biotin, vitamin E
34	Fin	Folic acid
35	Fragmentation: Erythrocytes	Biotin, vitamins B_{12} and E
36	Gasping, rapid	Pyridoxine
37	Goitre	Iodine
38	Growth, poor	Biotin, calcium, choline, energy, fat, folic acid, inositol, niacin, pantothenic acid, protein, pyridoxine, riboflavin, thiamine, vitamins A, B_{12}, C, D, and E
39	Hematocrit, reduced	Iron, vitamins C and E
40	Hemoglobin, low	Iron, vitamins B_{12} and C
41	Hemorrhage: Eye	Riboflavin, vitamin A
42	Gill	Vitamin C
43	Kidney	Choline, vitamins A and C
44	Liver	Vitamin C
45	Skin	Niacin, pantothenic acid, riboflavin, vitamins A and C
46	Irritability	Fatty acids, pyridoxine, thiamine
47	Lesion: Colon	Biotin, niacin
48	Eye	Methionine, riboflavin, vitamins A and C, zinc
49	Skin	Biotin, inositol, niacin, pantothenic acid
50	Lethargy	Folic acid, niacin, pantothenic acid, thiamine, vitamin C
51	Lipoid liver	Fatty acids, rancid fat
52	Lordosis	Vitamin C
53	Myopathy, cardiac	Essential fatty acids

continued

Table 7. Concluded.

Code	Symptom	Possible nutrient deficiencies
54	Necrosis, liver	Pantothenic acid
55	Nerve disorder	Pyridoxine, thiamine
56	Pale liver (glycogen)	High digestible carbohydrate, biotin
57	Photophobia	Niacin, riboflavin
58	Pinhead	Starvation
59	Pigmentation, iris	Riboflavin
60	Prostration	Pantothenic acid, vitamin C
61	Rigor mortis, rapid	Pyridoxine
62	Scoliosis	Phosphorus, tryptophan, vitamins C and D
63	Shock syndrome	Essential fatty acids
64	Slime, blue	Biotin, pyridoxine
65	Spasm, muscle	Niacin
66	Swimming, erratic	Pyridoxine, pantothenic acid
67	Tetany, white muscle	Niacin, vitamin D
68	Vascularization, cornea	Riboflavin

Note: For more details on all deficiency symptoms, refer to NRC (1981, 1983).

Table 8. Recommended nutrient levels in salmonid diets.

		Recommended level	Deficiency symptom codes[a]
Vitamins[b]			
Fat soluble			
Vitamin A	(IU/kg feed)	3500	03, 18, 25, 28, 29, 41, 43, 45, 48
Vitamin D_3	(IU/kg feed)	3000	62, 67
Vitamin E	(IU/kg feed)	100	01, 03, 10, 24, 26, 29, 31, 33, 35
Vitamin K	(mg/kg feed)	10	01, 13
Water soluble	(mg/kg feed)		
Ascorbic acid		300	01, 03, 08, 22, 29, 44, 50, 52, 60
B_{12}		0.02	01, 35, 40
Biotin		0.4	06, 14, 15, 19, 31, 33, 35, 47, 64
Choline		3000	31, 43
Folic acid		5	01, 14, 34, 50
Inositol		400	01, 23, 31, 49
Niacin		150	01, 25, 45, 47, 49, 50, 57, 65, 67
Pantothenic acid		60	04, 05, 12, 20, 30, 49, 54, 60, 66
Pyridoxine		10	04, 14, 15, 27, 36, 55, 61, 64, 66
Riboflavin		20	09, 11, 14, 28, 41, 48, 57, 59, 68
Thiamine		10	06, 09, 15, 16, 25, 27, 46, 50, 55
Minerals[c]			
Calcium	(g/kg feed)	0.2–0.3	32, 38
Phosphorus, inorganic	(g/kg feed)	7–8	17, 62
Magnesium	(g/kg feed)	0.5–0.7	02, 07, 38
Copper	(mg/kg feed)	3	38
Manganese	(mg/kg feed)	12–13	38
Selenium	(mg/kg feed)	0.1–0.4	21, 24
Zinc	(mg/kg feed)	15–30	09, 11, 28, 48
Iodine	(μg/kg feed)	0.6–1.1	37
Iron	(μg/kg feed)	Required	01

[a] Refer to Table 7.
[b] Based on NRC (1981) and practice in Fish Nutritional Laboratory, Ontario Ministry of Natural Resources.
[c] Lall (1981).

Thiamine

Thiamine functions as a coenzyme in carbohydrate metabolism, where it is required for the oxidative decarboxylation of pyruvate and the transketolation reaction in the pentose phosphate pathway. Deficiency symptoms include anorexia, poor growth, depigmentation, and high mortality. The thiamine requirement of channel catfish is 1 mg/kg dry diet and of salmonids is 10–12 mg/kg dry diet.

Riboflavin

Riboflavin is a component of flavin adenine dinucleotide, a coenzyme for several enzymes, including glutathione reductase and D-amino acid oxidase. Flavin mononucleotides also perform a number of metabolic functions. D-amino acid oxidase activity in rainbow trout liver was depressed by riboflavin deficiency and appeared to provide a sensitive indicator of incipient vitamin insufficiency. Riboflavin deficiency in salmonids led to anorexia, poor growth, cataracts, and increased mortality. The riboflavin requirement of salmonids is about 20 mg/kg diet.

Pyridoxine

As pyridoxal phosphate, this vitamin plays an important role in amino acid metabolism, being a coenzyme for all aminotransferases. Deficiency leads to erratic and rapid swimming, hyperirritability, poor growth, and high mortality. The pyridoxine requirement is generally in the range of 10–20 mg/kg diet.

Niacin

As a component of nicotinamide adenine dinucleotide and its phosphate, this vitamin is essential to all dehydrogenase reactions. Its absence from the diet leads to hemorrhage and skin lesions together with other nonspecific symptoms. The requirement level is in the range of 50–100 mg/kg diet for different species.

Pantothenic Acid

Pantothenic acid is a component of coenzyme A. This coenzyme is capable of accepting 2-carbon units, thus forming acetyl-coenzyme A, and it plays a unique role in many biological syntheses and degradations. In many species of fish, pantothenic acid deficiency results in mucous-covered gills, anorexia, reduced weight gain, and "clubbed" gills. Fingerlings of channel catfish and carp require 10 mg/kg diet and 40 mg/kg diet respectively.

Ascorbic Acid

An important antioxidant, ascorbic acid is involved in hydroxylation of proline and lysine during collagen formation. Deficiency leads to spinal deformities, e.g., scoliosis and lordosis. The requirement is in the range of 100–150 mg/kg diet for salmonids and 30–50 mg/kg diet for carp and channel catfish.

Choline

Choline serves as a source of methyl groups and is involved in a number of transmethylations; as phosphatidyl choline (lecithin), it has an important structural role in biomembranes. Deficiency of choline leads to fatty livers, anorexia, poor growth, and hemorrhaging of the kidney and intestine. The dietary requirement of salmonids for choline is about 800 mg/kg diet.

Folic Acid

Folic acid has an important metabolic role in 1-carbon transfers and is essential, therefore, for many metabolic interconversions. Deficiency leads to macrocytic normochromic anaemia in salmonids; other generalized symptoms include poor growth, anorexia, and lethargy. The requirement for folic acid is 5–10 mg/kg diet.

Cyanocobalamin

Cyanocobalamin functions as an integral part of the cobamide enzymes. Deficiency leads to macrocytic anaemia in salmonids; poor growth has also been reported in several other species of fish. The requirement for cyanocobalamin is 0.01–0.02 mg/kg diet.

Biotin

Biotin serves as a coenzyme in carboxylation reactions, two of the best known being pyruvate carboxylase (pyruvate to oxaloacetate, an important anaplerotic reaction) and acetyl-coA carboxylase, which is involved in the synthesis of fatty acids. In salmonids, the requirement for biotin is very low (0.1–0.5 mg/kg diet) and it is often difficult to induce biotin deficiency. No well-defined symptoms of biotin deficiency have been described.

Inositol

Inositol is, among other things, a component of one of the phosphatidyl phosphatides and, although phosphatidyl inositol is quantitatively a minor component of fish phospholipids, it appears to play an important role in the membrane function. The requirement levels are 200–400 mg/kg diet.

Vitamin A (Retinol)

Vitamin A has a role as a visual pigment; it may also be necessary for the maintenance of mucous membranes. No underlying biochemical lesion is known to explain the deficiency symptoms, which include exophthalmia, cataracts, abnormal cartilage, dermal depigmentation, and atrophy. The requirements for vitamin A are 1000–2000 IU/kg diet.

Vitamin D (Cholecalciferol)

A requirement for cholecalciferol has been demonstrated in channel catfish and rainbow trout. It is required, as in mammals, for bone mineralization and calcium homeostasis. Between 1600 and 2000 IU cholecalciferol/kg dry diet is the requirement for rainbow trout.

Vitamin E (Tocopherol)

Vitamin E is a lipid-soluble antioxidant that is located in biomembranes, i.e., cell membranes, mitochondria, reticulo-endothelial system. These structures contain large amounts of highly unsaturated fatty acids that are especially vulnerable to free radical initiated chain reactions. Tocopherol terminates such reactions. The requirement for tocopherol varies with polyunsaturated fatty acid levels in the diet but is 30–50 mg/kg diet in rainbow trout. Deficiency led to exudative diathesis and muscular dystrophy in Atlantic salmon; muscular dystrophy occurs in vitamin-E deficient carp.

Vitamin K

Absence of vitamin K in the diet induces extended blood clotting in tissues and hemorrhaging of the tissues following contusions in salmonids. Some doubt exists over whether or not channel catfish have a requirement for vitamin K.

Minerals

About 20 inorganic elements are required to maintain the structural and metabolic functions of vertebrates. Mineral metabolism differs from that of other nutrients in that, in contrast to proteins, carbohydrates, fats, and vitamins, minerals are neither produced nor consumed by the organism. For many vertebrates, uptake of minerals from the food can be regulated to only a limited extent. Nevertheless, most species have the ability to keep the concentration of ions constant in the body fluids, thus maintaining a constant internal milieu. This is achieved mainly by regulating excretion.

In fish, salt regulation is of special significance. Freshwater fish are likely to lose ions to the greatly hypotonic environment and thus suffer from hydration. The opposite is true of saltwater fish. Salt regulating mechanisms are highly developed in fish, especially those that migrate from freshwater to saltwater and vice versa. These mechanisms must make some demands on the energy budget of the fish. In contrast with mammals and birds, which derive most of the inorganic elements they require from the diet, with ingested water contributing to only a limited extent, the water environment may contribute substantially to the mineral requirements of fish.

Elements known to be required by fish are the bulk elements, i.e., calcium, chlorine, magnesium, phosphorus, potassium, and sodium, together with a number of trace elements. The latter include cobalt, copper, iodine, iron, manganese, selenium, and zinc; other trace elements that may be required include aluminum, chromium, and vanadium. It is extremely difficult to conduct experiments on mineral requirements because of the problem of limiting their concentration in the diet and especially because there will almost always be a waterborne contribution to the intake of any mineral. This is particularly true of the trace elements.

The greatest contribution of the environment to the mineral requirements of fish occurs, of course, in marine fish. After detailed experiments, Yone and Toshima (1979), in Japan, were able to show that purified diets for red sea bream need only be supplied with iron, potassium, and phosphorus to meet the mineral requirements of the fish; the remainder of the elements coming from the external environment. Their mineral mixture contained

5.2% KCl, 30.8% $NaH_2PO_4 \cdot 2H_2O$, and 1.5% Fe citrate (together with 62.5% alpha-cellulose). It was used at a level of 8% in the diet. Other marine fish seem unlikely to be any more demanding in their dietary mineral requirements than red sea bream.

Freshwater fish, on the other hand, require a mineral supplement in their food; some recommended levels are presented in Table 8. Many of the practical diets currently in use with salmonids contain a relatively high proportion of fish meal, which supplies the bulk (major) elements.

Certain interactions occur between minerals and these may complicate attempts to assess dietary requirements. These interactions include both antagonism and synergism, e.g., large amounts of calcium in a diet may lower the availability of dietary zinc, perhaps because the elements compete for the same binding sites or absorption mechanism. Certainly, zinc-deficiency symptoms may appear in salmonids because of a very high ratio of dietary calcium to zinc. Another example of interplay between elements is the occurrence of calcium deposition (nephrocalcinosis) in magnesium-deficient fish.

Minerals have a role in many facets of metabolism, such as hormones, respiratory pigments, structural elements, high-energy bonds, and metalloenzymes. The consequences of mineral imbalance or deficiency, therefore, may be very far reaching.

Calcium and Phosphorus

Calcium and phosphorus are frequently seen as being closely related because of their combined role in bone mineralization. In fact, the requirement of salmonids for phosphorus is higher than that for any other inorganic element and this requirement is not generally affected by dietary calcium levels. In controlled experiments, the growth of both carp and rainbow trout has been shown to be positively correlated with dietary phosphorus levels but not with calcium levels. By and large, it has been difficult with a number of fish species to bring about calcium deficiency, mainly because of their ability to absorb calcium from the water via the gills. The availability of inorganic phosphorus depends on the solubility of the salt concerned — the more soluble the salt the more available the phosphorus. Thus, the phosphorus available from tri-calcium phosphate is less than that available from mono- and di-calcium phosphate, especially in the case of stomachless fish.

Dietary phosphorus requirements have been reported as 0.4–0.47% for channel catfish, 0.6% inorganic phosphorus supplemented in a diet containing 0.7% phosphorus from plant sources for Atlantic salmon, 0.68% for red sea bream, 0.6–0.7% for common carp, and 0.7–0.8% for rainbow trout.

Calcium- and phosphorous-deficiency symptoms in channel catfish include reduced growth, poor feed efficiency, low bone ash, and low haematocrit levels. Red sea bream deficient in phosphorus contained lower vertebral ash, calcium, and phosphorus and had a more brittle bone structure. Common carp and rainbow trout given diets deficient in phosphorus had reduced calcium, phosphorus, and ash contents throughout their entire body and vertebrae.

Phosphorus is present in white fish meal as tri-calcium phosphate and the low availability of this compound in carp has led to its release into pond

water via the feces. There, it is slowly redissolved and together with the ammonia excreted by the carp can lead to extensive eutrophication in ponds. No satisfactory solution for controlling the pollution and, consequently, phytoplankton production that results, and at the same time providing a diet for carp that is suitably supplemented with phosphorus, appears to have been reached.

Magnesium

In addition to being a component of bone, magnesium occurs in many metalloenzymes and during magnesium deficiency many metabolic functions are affected. Apart from general symptoms (e.g., reduced weight gain and poor food conversion), magnesium deficiency in rainbow trout leads to renal calcinosis and a flaccidity of the muscle, due in part to an increase in extracellular fluid volume. Magnesium requirements for rainbow trout are 0.06–0.07% and for carp 0.04–0.05%.

Zinc

Zinc is also a component of metalloenzymes (superoxide dismutase, carboxypeptidase); thus, many metabolic functions are affected by a deficiency of zinc. In rainbow trout, zinc requirements are normally met by dietary levels of 15–30 mg/kg diet, although, as indicated earlier, larger amounts may be required to prevent calcium antagonism under certain circumstances. Dietary zinc levels of up to several hundred milligrams per kilogram diet do not seem injurious to rainbow trout. Zinc deficiency, on the other hand, manifests itself in the form of cataracts and other general symptoms (Fig. 2).

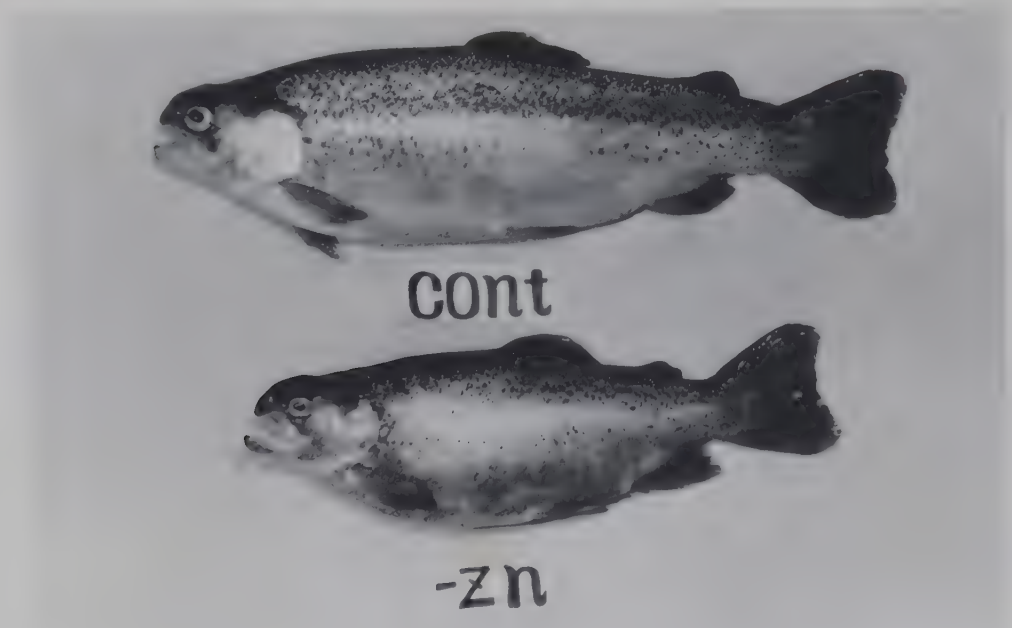


Fig. 2. Deficiency of zinc. Deletion of zinc from the mineral mixture in white fish meal diet resulted in short-body dwarfism, 80%; lens cataract, 100%; and no fin erosion (Satoh et al. 1983).

Iron

Dietary iron is essential to maintain normal haemoglobin content, haematocrit value, and mean corpuscular diameter. A minimum dietary iron concentration of 150 mg/kg is required to prevent iron-deficiency symptoms such as hypochromic, microcytic anaemia and anisocytosis in red sea bream and common carp.

Copper

Fingerlings of common carp given diets containing 0.7 mg copper/kg diet had lower weight gains than those given 3.0 mg copper/kg diet. In contrast, differential growth responses to different levels of copper (less than 1 mg/kg diet as opposed to 3 mg/kg diet) were not observed in rainbow trout. Copper requirements for channel catfish, if they exist at all, do not exceed 1.5 mg/kg dry diet. Copper concentrations of 20–30 mg/kg diet at a waterborne copper level of 0.33 g/L led to reductions in weight gain when compared with lower (3.5 mg/kg diet) dietary copper levels. Growth of rainbow trout was not affected by dietary copper concentrations of this magnitude.

Manganese

Manganese deficiency in rainbow trout gives rise to abnormal curvature of the backbone and malformation of the tail. Higher growth rates of both rainbow trout and carp were obtained when they were given diets with a manganese content of 12–13 mg/kg diet than when the manganese level was 4 mg/kg diet.

Selenium

Selenocysteine is a component of the metalloenzyme glutathione peroxidase. As such, it contributes to the antioxidant defence mechanisms of the fish, utilizing both hydrogen peroxide and organic hydroperoxides as substrates. Dietary selenium and vitamin E function synergistically in the prevention of oxidative damage. Selenium deficiency exacerbates vitamin-E deficiency symptoms, a double dietary deficiency leading to the rapid onset of muscular dystrophy and exudative diathesis. Selenium-deficiency symptoms could not be induced in rainbow trout at a dietary selenium level of 0.07 mg/kg diet (waterborne selenium 0.4 g/L, dietary vitamin E concentration 400 IU/kg diet), the lowest level attainable. Maximal glutathione peroxidase activity occurred at dietary selenium levels between 0.15 and 0.38 mg/kg. Selenium toxicity (uncoordinated spiral swimming behaviour 12–24 hours before death, reduced growth and feed efficiency) occurred at a dietary selenium concentration of 13 mg/kg diet.

Iodine

Iodine has a role in thyroid metabolism and the dietary iodide requirement of chinook salmon fingerlings has been based on the iodide content of the thyroid gland, amounting to 0.6 mg iodide/kg dry diet. Higher levels are recommended for periods in the life cycle when thyroid activity is thought to reach a peak. Thus, 1.1 mg iodide/kg diet is recommended for advanced parr, smoltification being presumed to be accompanied by increased thyroid activity.

Fish Feeds and Their Quality

Types of Fish Feed: Dry or Moist Diets

There are several forms of fish feed: moist pellets, steam treated or extruded dry pellets, and natural materials such as ground liver, spleen, lung, heart, and raw fish (wet feed). However, only two basic types of formulated (artificial) feed need to be considered for intensive fish culture — dry or moist feeds. Wet feed leads to water quality and pollution problems, caused by the feed breaking up and dissolving, and is expensive in terms of labour and freezer space. In spite of its desirable floating and water-stable characteristics, however, which enable one to observe feeding activity and thus avoid overfeeding, extruded (expanded) feed may be of inferior nutritive quality because of overheating during the manufacturing process. Dry and moist diets are similar in many ways, the basic difference being that moist pellets contain a high level of raw fish or slaughterhouse offals and by-products, which give the final product a high moisture level.

In most practical circumstances, the important factor for selecting feed for fish culture is not whether it is moist or dry feed because most fish feed formulations employed today can support reasonable growth without causing any physiological impairment or heavy mortality. The main causes of serious diet problems, on the other hand, are (1) the quality of feedstuffs used in the formula, (2) poor physical quality of granules and pellets, (3) deterioration of the feed due to improper storage conditions, and (4) poor husbandry practices.

Moist diets contain 25–35% water, contributed mainly by pasteurized whole fish, cannery by-products, and, sometimes, packinghouse by-products. The meal portion (40–60%) in the moist diet consists of dry diet ingredients such as fish meal, animal by-product meals, plant by-product meals, oil, binders, preservatives, and vitamin premix. Moist pelleted feed has several disadvantages in comparison with steam pelleted dry feed. Because of the high water content of this type of feed, it must be transported and stored in a freezer until used to avoid spoilage. As well, it may be difficult to obtain a regular supply of fresh raw fish and it is also possible that the fresh raw fish used may introduce some pathogens into the fish if not pasteurized. Improper transportation and storage damages or destroys certain vitamins and fats and increases fungal and bacterial growth in such feeds. Thus, the handling of moist feeds in a fish culture station is laborious and expensive.

Moist feeds have some merit in coastal regions where fresh raw fish and by-products and labour are regularly available and may have a significant economic advantage. It is also possible that the physical and chemical characteristics of moist pellets are more palatable to some fish species. There is no evidence, however, that such diets are nutritionally superior to dry feeds (Bromley 1980).

In contrast with moist diets, dry feeds are easier to manufacture, transport, and store. Bulk purchase of quality feed ingredients is possible, thus assuring a continuous supply of quality feed. The dry ingredients on the commodity market are more quality defined than raw fisheries products and can be supplied regularly. Hence, it is possible to formulate dry feeds more precisely using the available knowledge of fish nutrition. Furthermore, this

Table 9. Practical diet formulae for salmonids.

Ingredient	Production		Test	Brood
	MNR-83S (kg)	MNR-83G (kg)	C202 (kg)	MNR-83B (kg)
Fish meal, herring/caplin (68% CP, 13% ash)	46	27	30	35
Feather meal, hydrolyzed (80% CP, 4% ash)	8	8	—	8
Soybean meal, solvent extracted, dehulled (48% CP)	9	10	17	9
Corn gluten meal (60% CP)	8	10	13	7
Brewer's dried yeast (45% CP, 7% ash)	5	5	—	5
Alfalfa meal (17% CP, 24% fibre)	—	—	—	6
Whey, spray dried (12% CP, 10% ash)	8.5	6	—	7
Wheat middlings (17% CP, 8% fibre)	—	20	25	14
Vitamin premix (VIT-8204)[a]	1.5	1	2	2
Mineral premix (MIN-8204)[a]	1	1	1	1
Fish oil with antioxidant	3	3	3	2
Fish oil with antioxidant sprayed on pellets/granules	10	9	9	4

Notes: CP = crude protein. All ingredients must be ground finer than 0.25 mm.
[a] Refer to Table 10.

will lead to the formulation of "least-cost" fish diets in the future. Most nutrients in dry feeds, including vitamins and unsaturated fats (stabilized with antioxidants), are stable at room temperature and, therefore, can be stored safely without freezing. Feeding of dry pellets, either by hand or using mechanical feeders, is also much simpler than that of moist feeds. The dry feed formulae that have been employed successfully for many years at the salmonid fish culture stations of the Ontario Ministry of Natural Resources, Canada, are presented in Table 9.

Fry that have difficulty in accepting dry feeds can be adapted gradually to such diets by using a modified moist diet, e.g., different amounts of raw fish or fresh liver can be mixed in a dry starter diet, pelleted in a meat grinder, and fed as a moist feed (formula C403, Table 11). The problem of acceptability of dry feeds by some fish species may also be solved by better feeding techniques and fish-culture management. The growth rate of fish fed on most well-manufactured dry feeds made of high quality feedstuffs is usually superior to that of fish reared on moist diets.

Fish nutrition is still in the developmental stage and, therefore, in contrast with domestic animal diets, fish diets (dry or moist) are not as precisely balanced for nutritional requirements. They are not formulated on the basis of the true bioavailability of ingredients in the feed. Hence, there is some wastage of nutrients due to the generous safety margin used by feed manufacturers. This may increase the oxygen requirements of the fish and

Table 10. Vitamin and mineral premixes for salmonid diets.

(a) Formula VIT-8204.

Ingredient	g/kg premix
Vitamin A (as acetate)	500000 IU
Vitamin D_3	300000 IU
Vitamin E (dl-alpha-tocopheryl acetate)	10000 IU
Vitamin K (menadione sodium bisulfate)	3
Vitamin B_{12}	0.003
Ascorbic acid	40
Biotin	0.05
Folic acid	1
Niacin	20
Pantothenic acid (as D-calcium salt)	15
Pyridoxine (as HCl salt)	3
Riboflavin	5
Thiamine (as HCl salt)	3
Choline chloride (50%)	300
Wheat middlings[a]	+
Total premix	1000 g

[a] Wheat middlings are added in sufficient quantities to bring the total premix to 1000 g.

(b) Formula MIN-8204.

Ingredient	g/kg premix
Copper (as $CuSO_4 \cdot 5H_2O$)	2.5
Iron (as $FeSO_4 \cdot 7H_2O$)	6.3
Manganese (as $MnSO_4 \cdot H_2O$)	8.6
Iodide (as KI)	0.8
Zinc (as $ZnSO_4 \cdot H_2O$)	14.4
Salt (99% NaCl)	300.0
Wheat middlings[a]	+
Total premix	1000 g

[a] Wheat middlings are added in sufficient quantities to bring the total premix to 1000 g.

biological wastes in aquatic systems. Feeding technique and strategy, therefore, must be considered as a very critical part of the daily activity at most fish culture stations.

Table 11. Practical moist diet for salmonids (formula C403).

Ingredients	Percentage
Fish meal, herring or caplin[a] (>68% CP, 13% ash)	48
Soybean meal (48% CP)	10
Corn gluten meal (60% CP)	12
Whey (12% CP)	7
Brewer's yeast (45% CP)	5
Vitamin premix (VIT-8204)[b]	2
Mineral premix (MIN-8204)[b]	1
Fish oil with antioxidant	15
Add:	
Raw fish (fresh, ground, pasteurized)	1 kg per 2 kg dry meal mix
Formic acid	6 g per 2 kg dry meal mix

[a] Refer to Table 12.
[b] Refer to Table 10.

Table 12. Quality standards of fish meal and oil required for salmonid diets.

Compound	Level
Fish meal	
Crude protein (%N × 6.25)	>68%
Lipid	<10%
Ash, total	<13%
Salt (NaCl)	<3%
Moisture	<10%
Ammonia-N	<0.2%
Antioxidant (sprayed liquid form)	200 ppm
Check heavy metals	
Steam processed, ground finer than 0.25 mm	
Fish oil	
Peroxide value	<5 meq/kg
Anisidine value	<10 meq/kg
Total pesticides	<0.4 ppm
PCBs	<0.6 ppm
Nitrogen	<1%
Moisture	<1%
Antioxidant (liquid)	500 ppm
Deaerate and mix antioxidant by bubbling nitrogen gas before storage in airtight container	
No fortification of any vitamins	

Selection of Feed Ingredients

Feedstuffs or ingredients used in animal feeds are composed mainly of natural products, as well as many of the by-products of processing or milling industries. The proportion in which these components are present differs between feeds; thus, strictly speaking, no two feeds are nutritionally alike.

In the everyday formulation of diets, feedstuffs with generally similar properties may be substituted one for another, and exchanges made within mixtures in accordance with market price, local availability, and composition. In making substitutions, particular regard is also paid to essential nutrient content and balance of the final diet and, to a degree, culturist preference. Thus, different proportions of ingredients are combined to achieve the desired nutrient balance (Table 13). It is, therefore, expedient to establish categories of feedstuffs within which substitutions are feasible because of similar nutritional properties (e.g., forages, roughages, and concentrates for animals). Such a classification, however, is not readily

Table 13. Combination (%) of protein sources to balance essential amino acids.

Ingredients	Crude protein	Methionine	Cystine	Lysine	Methionine + cystine : lysine ratio
Soybean meal	47	0.7	0.7	3.2	0.4
Corn gluten meal	60	1.9	1.1	1.0	3.0
Soybean meal (90%) + corn gluten meal (10%)	49	0.8	0.8	3.0	0.5
Herring meal	70	2.2	0.7	5.7	0.5

applicable to fish-diet formulation because most of the feedstuffs used in fish feeds are derived from concentrates (e.g., fish meal, feather meal, soybean meal, and corn gluten meal).

Fish-feed concentrates can be categorized as follows: (1) fisheries by-products: fish meals, fish solubles; (2) animal by-products: meat meals, feather meals, blood meal; (3) dairy by-products: whey, skim milk, milk powder; (4) plant protein by-products: soybean meal, corn gluten meal, coconut meal, linseed meal, peanut meal; (5) fermentation by-products: brewer's dried yeast, corn fermented extracts; (6) single cell/algae products; (7) grain by-products: wheat middlings, ground corn, wheat and oats, tapioca meal, rice bran; (8) oils and fats: fish oil, plant oils, animal fats; and (9) supplements and additives: vitamins, minerals, binders.

These concentrates make up 50–80% of fish diets, fish meal comprising at least 20–50% of salmonid diets. Fish oil makes up 5→20% of the diet and the remainder is composed of milling by-products of grain, such as wheat middlings, consisting mainly of starch and playing an important role as a binder rather than energy source in salmonid diets. Thus, much of the quality of feed for carnivorous fish is dependent upon the quality of the fish meal and fish oil.

Acceptability or palatability of feeds is also dependent upon the selection of ingredients. Higher levels of fish meal and fish oil in the diet improve the acceptability of feed, leading to increased feed intake. On the other hand, plant protein ingredients, such as soybean meal, influence the acceptability of feed for young fish and at higher levels of inclusion (>30%) reduce feed intake significantly, particularly in carnivorous fish, despite its highly digestible nutrient content.

Pelletability of feed and its durability are affected by the physical characteristics of the ingredients in the formula. Several kinds of binder are available in the market and these may improve pelletability and durability but the main effect is still dependent on the amount and type of milling by-products containing high levels of starch, which is gelatinized during the pelleting process.

Another consideration in selecting feed ingredients is variability of the feedstuffs related to the source of the plant, animal, or fisheries products; season of the year; and processing methods. The lower the variability of the product, the more reliance can be placed on its quality — a very desirable factor in the selection of ingredients for the diet. Thus, selection of ingredients based on composition, nutrient digestibility, acceptability, pelletability, availability, and price are the critical considerations in the manufacture of a quality feed for fish.

Ingredient Quality and Digestibility

Diets produced from poor raw materials and under adverse processing conditions have adverse effects on fish health. Many of the feed ingredients employed in fish diets are also used by the animal-feed market and, therefore, information about their average quality and composition is available from feed mills and feed brokers (Table 14). In most cases, the main emphasis of quality control for fish diets is on fish meals and fish oils, which between them constitute 20–50% of fish rations. There are various qualities of fish meals and oils on the market. These relate to the quality of

Table 14. Proximate composition (%) of common feed ingredients in Asia.

Ingredient	Water	Protein	Fat	Fibre	Ash	Lysine	Methionine + cystine
Buttermilk	7.0	32.0	5.0	0.1	8.0	2.4	1.1
Fish meal (1)	9.5	53.9	6.1	3.0	25.7	3.7	1.7
Fish meal (2)	10.7	55.0	6.7	2.8	28.0	5.1	2.8
Meat meal	70	46.0	8.8	2.8	32.5	2.2	1.2
Skim-milk powder	9.6	33.0	1.0	2.0	7.6	2.6	1.4
Whey powder	8.0	12.0	0.8	0.2	9.0	1.0	0.5
Brewer's yeast	8.1	26.0	12.1	0.9	10.9	0.8	1.0
Yeast, dried	8.9	44.2	1.4	3.0	6.8	3.0	1.2
Groundnut meal	9.4	42.0	1.5	11.8	7.0	2.0	1.0
Palm kernel meal	8.9	14.0	2.6	17.4	3.8	1.0	0.6
Sesame meal	7.4	37.4	6.9	6.5	13.1	1.2	1.8
Soybean meal	10.8	43.0	2.5	6.6	6.5	2.8	1.3
Maize	11.0	8.5	3.6	2.5	1.3	0.2	0.3
Rice bran (1)	10.0	8.5	8.0	20.0	14.2	0.6	0.4
Rice bran (2)	9.7	12.5	1.3	16.0	13.0	0.5	0.3
Sorghum	13.1	7.6	2.5	2.5	1.7	0.3	0.3
Tapioca meal	11.5	2.1	0.5	3.0	9.4	—	—
Wheat bran	11.6	14.0	3.0	8.9	4.4	0.4	0.3
Wheat grain	11.8	11.7	1.7	2.8	1.6	0.3	0.3
Fish oil	1.0	—	99.0	—	—	—	—
Red palm oil	1.0	—	99.0	—	—	—	—

the original raw fish, processing techniques, storage methods, level of ash in the meals, and concentration of environmental contaminants in both fish meals and oils. The desirable quality standards for fish meals and oils used in the manufacture of salmonid diets are shown in Table 12 (Cho 1983). All other ingredients should also be monitored for quality, composition, toxins, antinutritional factors, contaminants, bacteria, fungus, and cross contaminants.

Fish have different digestive capabilities from terrestrial animals. Many feedstuffs, particularly cereal grains and grain by-products, which contain high levels of starch, are poorly digested by carnivorous fish. Therefore, the amount of digestible energy, and protein in particular, should be calculated based on the digestibility coefficients determined for a particular species of fish. The composition and digestibility values of the more important ingredients for salmonid diets are given in Tables 15 and 16.

As shown in Table 14 and Fig. 3, the proximate composition of all ingredients must be known, and other analyses (Table 12) carried out prior to or immediately after purchase. Any ingredient with high ash or crude fibre content should be treated with caution and used sparingly in the diet. No fish can utilize very much ash or fibre in natural ingredients, including grass carp. Even the degree of utilization of raw starch by herbivorous fish is controversial. Thus, the biological and chemical qualities of the feed ingredients in a diet, and the balance of biologically available nutrients, are much more critical in the production of a nutritionally sound and productive diet than is the use of any "magical" formula.

Table 15. Composition (%) of common ingredients in fish diets.

Ingredient	International feed number	Crude protein	Crude fat	Crude fibre	Ca	Methionine + cystine
Alfalfa meal	1-00-023	17.0	3.0	24.0	1.3	0.5
Blood meal, animal	5-00-381	80.0	1.0	1.0	0.3	2.4
Brewer's dried yeast	7-05-527	45.0	0.4	1.5	0.1	1.5
Corn, yellow	4-02-935	8.9	3.5	2.9	0.01	0.3
Corn gluten feed	5-02-903	21.0	2.0	10.0	0.3	1.0
Corn gluten meal	5-09-318	60.0	2.0	2.5	0.02	3.0
Corn distiller's dried soluble	5-02-844	27.0	9.0	4.0	0.4	1.2
Feather meal, poultry	5-03-795	85.0	2.5	1.5	0.2	3.6
Fish meal, herring	5-02-000	70.0	10.0	1.0	2.0	2.9
Meat and bone meal	5-09-321	45.0	8.5	2.5	11.0	0.8
Poultry by-product meal	5-03-798	58.0	14.0	2.5	4.0	2.0
Rapeseed meal	5-03-871	36.0	2.6	13.2	0.7	1.2
Soybean, full fat, cook	5-04-597	38.0	18.0	5.0	0.3	1.1
Soybean meal, dehull	5-04-612	48.0	0.5	3.0	0.2	1.5
Wheat middlings	4-05-205	17.0	3.6	7.0	0.2	0.3
Whey, dehydrated	4-01-182	12.0	0.7	—	0.9	0.5
Fish protein concentrate		80.0	0.3	1.0	3.5	4.7
Soybean protein concentrate		68.0	0.3	3.6	0.3	2.8
C201-Guelph reference diet		40.0	15.0	3.4	0.8	1.6
C202-Guelph reference diet		40.0	17.0	3.0	0.7	1.7

Note: For complete composition of various feedstuffs, refer to NRC (1981, 1983).

Table 16. Apparent digestibility coefficients and digestible energy values of ingredients in diets fed to rainbow trout.

Ingredient	International feed number	Dry matter (%)	Crude protein (%)	Lipid (%)	Gross energy (%)	Digestible energy (MJ/kg)
Alfalfa meal	1-00-023	39	87	71	43	7.7
Blood meal, animal						
Spray dried	5-00-381	91	99	—	89	19.4
Flame dried	5-00-381	55	16	—	50	10.0
Brewer's dried yeast	7-05-527	76	91	—	77	13.9
Corn, yellow	4-02-935	23	95	—	39	5.0
Corn gluten feed	5-02-903	23	92	—	29	5.4
Corn gluten meal	5-09-318	80	96	—	83	17.6
Corn distiller's dried soluble	5-02-844	46	85	71	51	10.7
Feather meal, poultry	5-03-795	75	58	—	70	15.7
Fish meal, herring	5-02-000	85	92	97	91	18.8
Meat and bone meal	5-09-321	78	85	73	85	16.0
Poultry by-product meal	5-03-798	52	68	79	71	13.9
Rapeseed meal	5-03-871	35	77	—	45	8.1
Soybean, full fat, cook	5-04-597	78	96	94	85	19.0
Soybean meal, dehull	5-04-612	74	96	—	75	13.5
Wheat middlings	4-05-205	35	92	—	46	7.0
Whey, dehydrated	4-01-182	97	96	—	94	16.0
Fish protein concentrate		90	95	—	94	17.2
Soybean protein concentrate		77	97	—	84	15.4
C202-Guelph reference diet		71	94	93	81	17.6

Notes: 1 MJ = 239 kcal. For composition of ingredients, refer to Table 15.

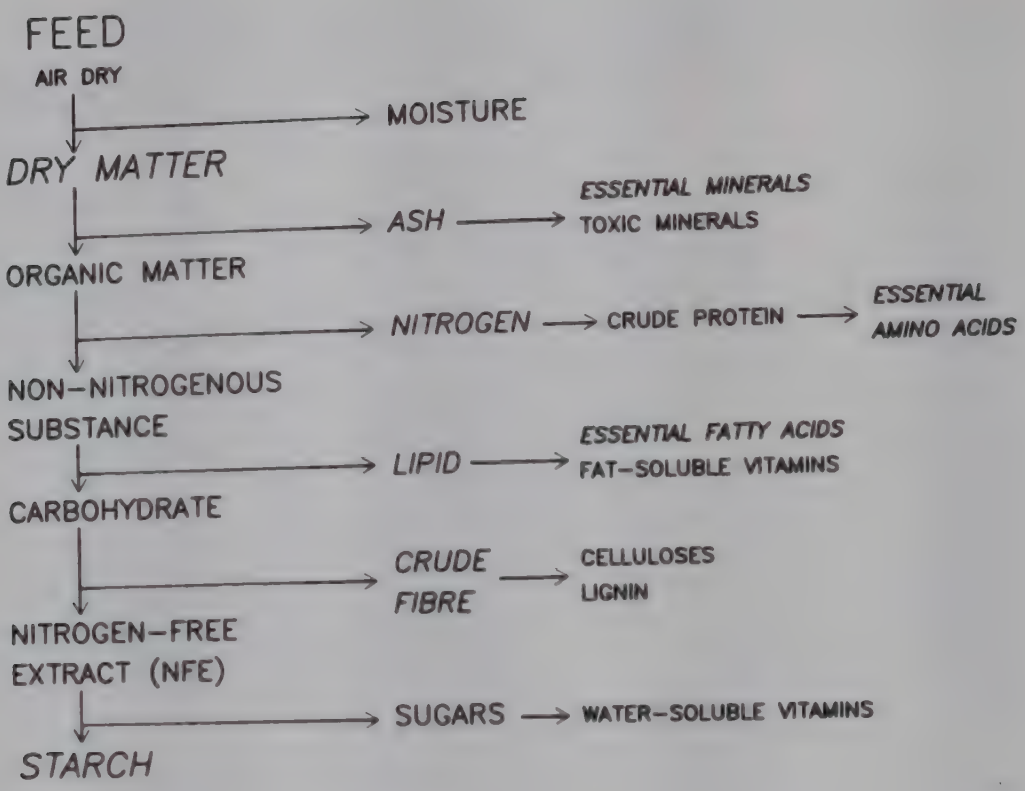


Fig. 3. The Weende proximate analyses.

Diet Formulation

The formulation of a diet represents the translation of energy and nutrient requirements into a balanced mixture of feed ingredients for a group of animals. This diet should then meet the daily need for energy and nutrients to support the maintenance and growth of the animal.

Fish are poikilothermic, however, and water temperature, therefore, has a profound effect on their metabolic needs. Thus, the correct balance of energy/nutrients at one temperature may not be appropriate for other temperatures.

The complexities of the effects of water temperature on the balance of energy and nutrient requirements has not yet been fully documented. Once these data are available, however, it may be possible to present the information as a matrix of energy requirements for each of a range of temperatures. Because fish species vary, it is to be expected that there might be a matrix for each species, although there is no evidence to indicate that the relative amounts of essential nutrients required by any species changes with temperature. Many feeding guides available for salmonid fish are "paper" conversions of feeding guides formerly used for beef liver and supplementary meal mixture feeding. The feeding standards must be based on the daily requirements of biologically available (digestible) energy and nutrients. Simplistically, the physical weight of feed based on live body weight (weight of feed as a percentage of body weight per day) does not have any merit compared with carefully practiced feeding to satiation.

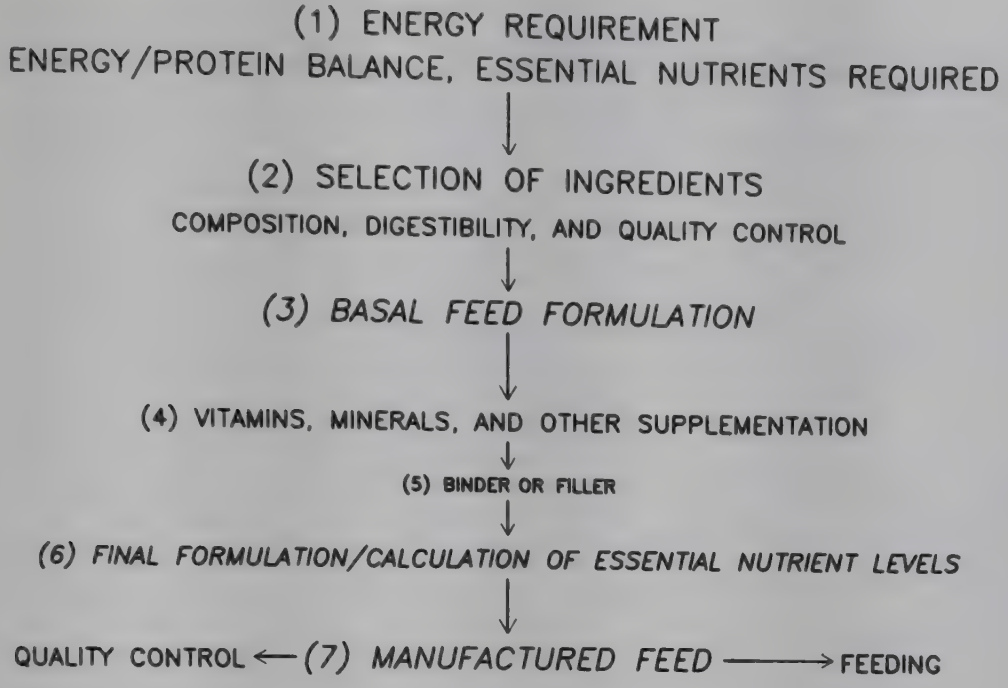


Fig. 4. Procedure for diet formulation.

Feeding guides relating weight of feed to body weight of fish may be misleading to the nutritionist because the nutrient density of the diet can vary widely, the energy density in particular being directly related to the level of fat in the diet. More realistic and practical feeding guides should be designed that indicate the amounts of "available" energy and other nutrients required each day, rather than providing only the weights of feed required.

The procedure for diet formulation is outlined in Fig. 4 and Table 17. First, the energy needs of the animal must be estimated from past records of growth; then, protein (amino acids) and other essential nutrient needs are estimated according to the energy content of the diet (step 1). Most of the dry carcass of salmonid fish contains 20–25 kJ/g (5–6 kcal/g) and 20–25 mg protein/kJ of energy. This is at least a starting point for estimating energy and nutrient needs if one tries to "guesstimate" expected body weight gain.

The next step is to select ingredients that are available locally and meet the characteristics required for the fish feed (step 2). The basal or fixed part of the feed can then be formulated using various sources of information. For example, it is desirable to include certain levels of fish meal in salmonid diets (25% in the example formulation), as well as soybean and corn gluten meals to balance essential amino acids (Table 13) and fish oil for essential fatty acids (step 3). Usually, fixed levels of vitamin and mineral premixes are also included, these being formulated separately. One percent of the diet is also reserved for any supplementation that may be needed after the final calculation of the nutrient content. If this is not required, it can be filled with any of the grain products without altering the balance of nutrients. The energy and protein content of the basal feed are then calculated. In the example in Table 17, 56 kg of basal feed is initially formulated out of the final feed weight of 100 kg. The basal feed (56 kg) contains a total of 1047 MJ digestible energy (18.7 MJ/kg) of the 1700 MJ

Table 17. Example of fish-diet formulation (see also Fig. 4).

Step 1: Nutrient requirements (e.g., salmonids)

Feed (kg)	Digestible energy (MJ)	Digestible protein (kg)	Methionine + cystine (kg)	Arginine (kg)
100	1700	35.0	1.6	2.4

(Protein/energy = 20 g protein/MJ)

Step 2: Selection of ingredients and their composition

Ingredient	Digestible energy (MJ/kg)	Digestible protein (%)	Methionine + cystine (%)	Arginine (%)
Fish meal	18.8	64.4	2.9	5.1
Soybean meal	13.5	46.1	1.5	3.4
Corn gluten meal	17.6	57.6	3.0	1.7
Wheat middlings	7.0	15.6	0.3	0.9
Fish oil	38.0	0	—	—
Vitamin premix	—	—	—	—
Mineral premix	—	—	—	—
Supplement premix	?	?	—	—

Note: All formulations should be calculated on a dry-matter basis.

Step 3: Basal or fixed part of formulation including vitamin, mineral, and supplement premixes

		Digestible energy	Digestible protein	
Fish meal	25	470	16.1	Minimum required
Soybean meal	10	135	4.6	Minimum required
Corn gluten meal	10	176	5.8	Minimum required
Fish oil	7	266	0	Minimum required

Step 4

Vitamins	2	0	0	Maximum required
Minerals	1	0	0	Maximum required
Supplement	1	?	?	
Basal, total	56	1047 (18.7 MJ/kg)	26.5 (47.3%)	
Other feeds need to contribute	44	653 (14.8 MJ/kg)	8.5 (19.3%)	(refer to step 6 for formulation)

Note: Vitamin and mineral premixes are designed to satisfy the requirements and fish oil with antioxidant supply all essential fatty acids

Step 5: Wheat middlings are used as both binder and filler in this formula

Step 6: Remainder of formula to be made up from wheat middlings (W), soybean meal (S), and fish oil (F) to meet the energy and protein requirements of the final diet

Simultaneous equations:

(1) W + S + F = 44 kg feed

continued

Table 17. Continued.

(2) $7.0\ W + 13.5\ S + 38\ F = 653$ MJ energy
(3) $0.156\ W + 0.461\ S + 0\ F = 8.5$ kg protein
(i.e., 7.0 = energy and 0.156 = protein contents of wheat middlings respectively)

Solution of equations:

From equation (1)
(4) $W = 44 - S - F$

Then substitute equation (4) into equations (2) and (3)
$7.0\ (44 - S - F) + 13.5\ S + 38\ F = 653$
$0.156\ (44 - S - F) + 0.461\ S + 0\ F = 8.5$

$308 - 7.0\ S - 7.0\ F + 13.5\ S + 38\ F = 653$
$6.86 - 0.156\ S - 0.156\ F + 0.461\ S + 0\ F = 8.5$

(5) $6.5\ S + 31\ F = 345$
(6) $0.305\ S - 0.156\ F = 1.64$

Therefore, from equation (5)
(7) $S = (345 - 31\ F)/6.5$

Then, substitute equation (7) into equation (6)
(8) $0.305\ ((345 - 31\ F)/6.5) - 0.156\ F = 1.64$

Therefore, from equation (8)
$F = 9.03$

Then, substitute equation (8) into equation (5)
$6.5\ S + (31 \times 9.03) = 345$
$S = 9.99$

Therefore,
$W = 44 - 9.99 - 9.03 = 24.98$

Answer: $W = 24.98$; $S = 9.99$; $F = 9.03$
Therefore, an additional 25 kg wheat middlings, 10 kg soybean meal, and 9 kg fish oil need to be added to the basal feed to meet energy and protein requirements

Note: Part of the fish meal may be replaced with ingredients from fisheries, animal, and dairy by-products; soybean meal and corn gluten meal with plant protein and fermentation by-products; wheat middlings with grain by-products; and fish oil with oils and fats, all of which have been categorized earlier as fish feed concentrates

Precaution: Improper substitution can result in nutritionally unbalanced modifications of diet formula and unsatisfactory palatability and pelletability. Knowledge of feeds, characteristics of ingredients, and nutritional judgment are required

Diet formula and composition:

	Feed (kg)	Digestible energy (MJ)	Digestible protein (kg)	Methionine + cystine (kg)	Arginine (kg)
Fish meal	25	~~4.70~~ 470	16.1	0.7	1.3
Soybean meal	20	~~2.7~~ 270	9.2	0.3	0.7
Corn gluten meal	10	~~1.8~~ 180	5.8	0.3	0.2

continued

Table 17. Concluded.

Wheat middlings	25	~~1.8~~ 180	3.9	0.1	0.2
Fish oil	16	~~6.7~~ 610	0	0	0
Vitamins	2	— –	—	—	—
Minerals	1	— –	—	—	—
Supplement[a]	1	? ?	?	?	?
Total	100	~~17.1~~ 1710	35.0	1.4[a]	2.4

[a] An additional 0.2% methionine should be supplemented to meet the requirement; therefore, the supplement mix is made with 0.2 kg methionine and 0.8 kg wheat middlings.

Note: Also check the levels of other essential amino acids.

Step 7: Mixing sheet is prepared for the amount of feed to be manufactured and samples of the ingredients and finished diet will be taken for the quality-control analysis

required and 26.5 kg (47.3%) of the 35 kg digestible protein required in the final feed. Therefore, the balance of the feed (44 kg) must contain 653 MJ digestible energy (14.8 MJ/kg) and 8.5 kg (19.3%) digestible protein (step 4). Wheat middlings was chosen as both binder and "filler" for steam pelleting (step 5). Although some of the ingredients can be substituted, the feed characteristics and balance of available nutrients must be retained.

From this point, using data extracted from steps 1 – 5 and using wheat middlings, soybean meal, and fish oil as ingredients, simultaneous equations 1 – 3 should be solved as shown in equations 4 – 8. Thus, the final diet formula and its composition are obtained (step 6). In the example in Table 17, the level of sulphur amino acids, methionine, and cystine was not met and, consequently, an additional 0.2% of these amino acids had to be provided using the spare supplement space. As a final step, it is necessary to check the contents of essential nutrients in the diet. A mixing sheet is then made for the feed-mill operator and samples of the ingredients, and also the finished product, are taken for quality-control analysis and future reference (step 7).

Vitamin and mineral premixes for fish diets are handled differently than those used in domestic animal diets. This is partly due to the lack of rigid requirements for different fish and because losses occur during manufacturing and storage. In addition, fish feeds are manufactured and used over long periods of time, whereas animal feeds are manufactured and used within a few days. Furthermore, the availability to fish of vitamins and minerals present in natural ingredients is not known and the contents measured using chemical and microbiological methods do not necessarily indicate the amount of vitamins and minerals available to fish. Premixes, therefore, generally supply most of the needs of the fish together with a "safety margin."

As shown in Table 12, antioxidants (e.g., ethoxyquin, BHT, and BHA) should be added to oil or feed to stabilize and prevent rancid oxidation of the dietary fats, particularly in tropical regions. Several formulae of practical and semipurified test diets for different fish are presented in Tables 9 – 11, and 18 – 21.

Computerized Least-Cost Formula Approach

Some "least-cost" formulae have been employed successfully within the commercial aquaculture sector for several years. As more knowledge on

Table 18. Practical diet formula for marine fish (formula C506).

Ingredients	%	% CP	% fat	% ash
Fish meal (65% CP; 20% ash)	50–60	32–39	3–4	12–14
Soybean meal, ground (44% CP)	15	7	1	1
Meat and bone meal, ground (50% CP)	10	5	1	3
Corn, tapioca, wheat or rice bran, ground	20–10	2	1	0
Vitamin premix (VIT-8204)[a]	1.5	0	0	0
Fish- or cod-liver oil with antioxidant	3.5	0	3	0
Total	100	46–53	9–10	16–17

Note: CP = crude protein.
[a] Refer to Table 10.

Table 19. Grower diet for carp (CM-80).

Ingredients	%	kg	Total (kg)
Fish meal	27	540	540
Soybean meal, ground	30	600	1140
Groundnut or other oil meal, ground	10	200	1340
Brewer's yeast	5	100	1440
Maize, wheat, tapioca or rice bran, ground	20	400	1840
Vitamin and mineral premixes	3	60	1900
Fish oil	5	100	2000
Total	100	2000	

Table 20. Purified test diet (formula C102).[a]

Ingredients	%
Casein, vitamin-free	40–(45)[b]
Gelatin	4
Starch	11–(16)[b]
Dextrin, white	9
D-glucose (cerelose)	5
Alpha-cellulose	3
Amino acid supplement (0.5% methionine; 1.0% arginine; 0.5% starch)	2
Vitamin premix (VIT-102)	3
Mineral premix (MIN-101)	8
Oil, marine with 0.05% antioxidant (or other oils as required)	15–(10)[b]
Total	100

[a] Control test diet that has been successfully employed for many years at the Fish Nutritional Laboratory, University of Guelph, Guelph, Ontario, Canada.
[b] Adjust protein or fat levels if necessary. Steam pellet at 5–10 psi without water.

digestibility coefficients and the limitations of various feed ingredients becomes available, the constraints (nutrient requirements) and upper and lower ingredient levels for least-cost feed formulation can be set more accurately. Economical diets could then be more readily formulated by computer for the various types of fish-husbandry practices and for different water temperatures. However, the requirements for the nutrients by fish and

Table 21. Vitamin and mineral premix for "purified diet."

(a) Formula VIT-102.

mg/kg feed	Vitamin
7000 IU	Vitamin A (acetate)
3000 IU	Vitamin D_3
200 IU	Vitamin E (dl-alpha-tocopheryl acetate)
50	Vitamin K (menadione sodium bisulfate)
40	Thiamine HCl
60	Riboflavin
200	D-calcium pantothenate
0.5	Biotin
20	Folic acid
0.2	Vitamin B_{12}
300	Niacin
40	Pyridoxine HCl
500	Inositol
500	Ascorbic acid
6000	Choline citrate
+	Alpha-cellulose or starch[a]
30000	Total (3% of diet)

[a] Alpha-cellulose or starch is added in sufficient quantities to bring the total premix to 30 g.

(b) Formula MIN-101.

mg/kg feed	Mineral
30000	$CaHPO_4 \cdot 2H_2O$ (23% Ca, 18% P)
3000	$CaCO_3$ (40% Ca)
15000	NaCl (39% Na)
20000	K_2SO_4 (45% K)
10000	$MgSO_4$ (20% Mg)
700	$FeSO_4 \cdot 7H_2O$ (21% Fe)
300	$MnSO_4 \cdot H_2O$ (33% Mn)
550	$ZnSO_4 \cdot H_2O$ (36% Zn)
160	$CuSO_4 \cdot 5H_2O$ (25% Cu)
26	$CoCl_2 \cdot 6H_2O$ (25% Co)
15	KI (76% I)
2.5	Na_2SeO_3 (42% Se)
+	Alpha-cellulose or starch[a]
80000	Total (8% of diet)

[a] Alpha-cellulose or starch is added in sufficient quantities to bring the total premix to 80 g.

the availability of these nutrients in different ingredients are not well known. Therefore, overemphasis on the least-cost formulation of fish diets could be premature in light of the present state of knowledge on fish nutrition.

The necessity of the least-cost approach will become more apparent as the scale of operation becomes larger, total production of feed from a feed mill becomes greater, and prices in the commodity market fluctuate more. The benefits of the least-cost formulation of fish feed are limited, however, relative to other animal feeds because a large proportion of the ingredient cost is tied to a few ingredients, such as fish meal and oil. Also, the choice of grain by-products, which are the major constituents of animal feeds, is limited in fish-feed formulae. The unit price of fish feed is much greater than that of animal feed; therefore, any saving in feed cost will help the

aquaculture industry in the future. For most fish-farm operations, 35–50% of the production cost is on feed, making it the single largest expense. Further development and testing of least-cost formulae for fish production will require greater cooperation from feed mills, which manufacture the feed, and fish farms, which test the feed under field conditions, and nutritionists, who carry out research to determine exact nutrient requirements of fish and the digestibility coefficients and optimum levels of the various ingredients used in fish feeds. In the meantime, more attention should be focused on feeding and husbandry practices to maximize utilization of the diet by the fish.

Feed Manufacturing and Its Specifications

A formulated dry fish feed must be pelleted or crumbled to be durable and water stable. The feed must also have the desirable physical and textural characteristics and correct particle size to be readily acceptable to different sizes of fish. Disintegrated and uneaten food pollutes the water and creates stresses, as a result of low oxygen, high nitrogen, and high organic matter, that have serious effects on the growth and health of the fish. Some of the important factors to be considered in manufacturing a durable dry fish feed without fines are physical properties and particle sizes of the ingredients, conditioning time and temperature in the pellet mill, quality of the steam supply, degree of compression through the die, and efficiency of sifting/grading and fat spraying. These processes take place through the complex system of the feed mill, illustrated in Fig. 5. Many of the problems experienced in fish culture in the past were related to the physical quality of the pellets and granules due to poor manufacturing processes and negligent

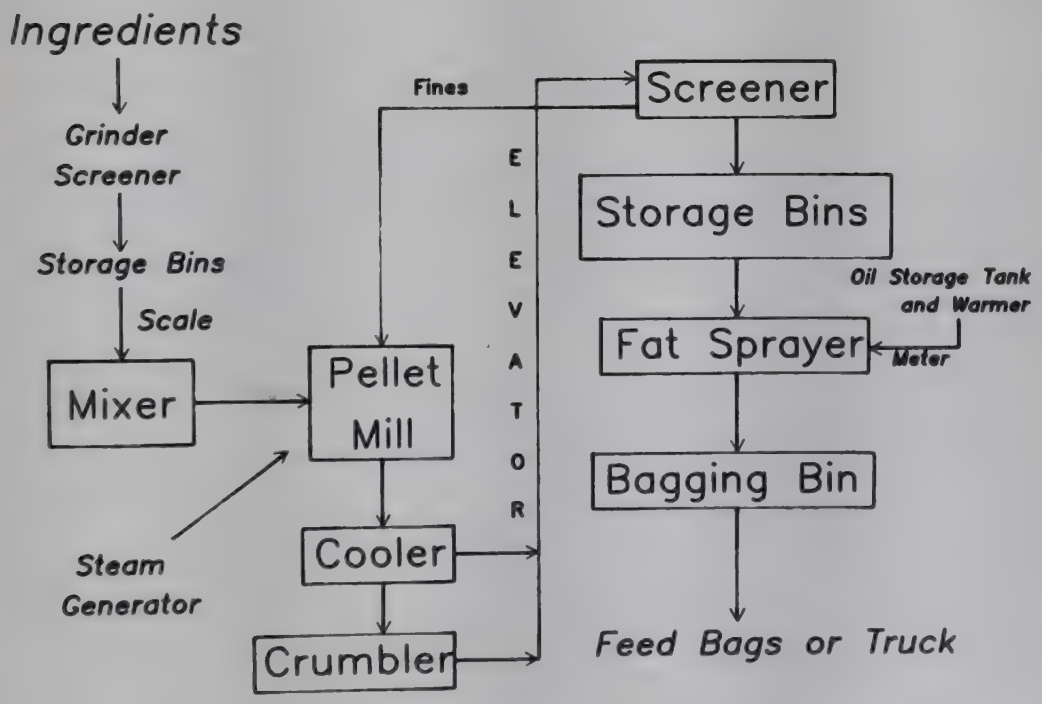


Fig. 5. Material flow in steam-pelleting process.

practices. For fish feed, the manufacturing process is of extreme importance. Supplying dietary nutrients to fish through the water medium presents problems that are unknown in animal-feeding practices. Therefore, a random sample of feed bags should be checked for the presence of excess fines, undersized granules, durability, foreign particles, too little or too much oil, and other evidence of poor quality. Any bag or batch of feed judged to be questionable should not be shipped to fish culture stations.

An example of the manufacturing specifications for salmonid fish feed that has been used for a number of years by the Fisheries Branch, Ontario Ministry of Natural Resources, Canada, for a contract with the Ontario Feed Manufacturers to supply feed for fish culture stations is presented below. These specifications have fulfilled a satisfactory role and have helped to improve the quality of fish feed in the Province of Ontario and have now also been applied to private markets.

There are three methods of manufacturing fish feeds: steam pelleting, extrusion, and dravo processing. Because a detailed description of these methods and of the structure and operation of the mills involved are beyond the scope of nutritionists, it is best to seek the expertise of the feed mills. The manufacturing of well-balanced diet formulae by nutritionists requires considerable cooperation with neighbourhood feed mills to transform the ingredients used in the feeds into nutritive diets for the fish.

Manufacturing Specifications for Fish Feed — An Example

The contractor's mill must comply with the Standards of the Registration SOR/83-593 Feeds Regulations, 1983, Canada Feed Act.

Equipment and Methods

Premix equipment: The mixer must be the batch type, with complete clean-out features. Premix equipment must include adequate scales to weigh microingredients in gram quantities.

Premix preparation: Premixes are used to facilitate dispersion of vitamins, minerals, and other trace materials that are normally required in small quantities. A premix must be finely ground (< 0.25 mm) and a uniform mixture of microingredients with a carrier of such physical properties that separation does not occur. A premix must consist of at least 0.5% of the entire mix and use an ingredient (e.g., whey, wheat middlings) contained in the diet as a diluent. The premix is then blended with a quantity of feedstuffs equal to 3–5% of the total mix. Introduction of this diluted premix into a batch system must be made midway in the loading of ingredients that comprise the formula.

If the premixes are supplied by a second company, this clause will apply to that company's manufacturing practices.

Manufacturing equipment: The feed mixer may be batch or continuous system, which must meet ASAE Standard: S303.1 (Agricultural Engineers Yearbook 1973), and all scales must meet the Canadian scale code.

Mixing, pelleting, and crumbling of feed: The following production records should be provided after completion of mixing: (1) formulation code, (2) ingredient identification and weight in each mix, (3) total weight of each mix, (4) date of mixing and batch number, and (5) measure of actual yield in bags or bulk of finished product.

Starter formula shall usually be made as 0.5, 1.0, or 1.5 mm granules. Grower formula shall usually be made as 2.0 mm granules or larger. When

Table 22. Feed sizes and feeding frequency.

Feed	Feed size	Standard sieve no.	Screen opening (mm)	Feedings per day	Fish size (g)
Brood stock pellets					
7 Pt	6.4 mm × 7 mm long	5/16" — #3.5	8 — 5.7	0.5–2	>200
Grower pellets					
6 Pt	6.4 mm × 6 mm long	5/16" — #3.5	8 — 5.7	1–2	>200
5 Pt	4.8 mm × 5 mm long	— #4	— 4.8	2	<200
4 Pt	3.4 mm × 4 mm long	— #6	— 3.4	3	100
3 Pt	2.4 mm × 3 mm long	— #8	— 2.4	3	50
Grower granules					
3 Gr	3 mm	#6 — #8	3.4 — 2.4	3	<50
2 Gr	2 mm	— #12	— 1.7	4	20
Starter granules					
1.5 Gr	1.5 mm	#12 — #16	1.7 — 1.2	4	<10
1 Gr	1 mm	— #25	— 0.7	5	3
0.5 Gr	0.5 mm	— #40	— 0.4	6–8	1

the particle size is greater than 3.0 mm, feed should be made in pellet form. Broodstock formula shall be made as 6.4 mm × 6.0 mm pellets or larger (Table 22). All ingredients shall be thoroughly pulverized (<0.25 mm) before mixing into the diets. The mix shall be processed into pellets using live, dry steam from a high-pressure boiler to make a pellet of the right texture that will be hard enough to hold together during packaging, transporting, and storing. When the pellets are extruded through a die, they must be moist enough to be spongy when pressed between the fingers. Shiny surfaced, flinty pellets are not of this type. The steam temperature in the die chamber must be approximately 90°C and pellets must be cooled immediately after pelleting.

Granules shall be manufactured by crumbling 4.0 or 5.0 mm pellets. "Fines" that result from the manufacture of pellets or granules of an intended size must not be used as part of the starter feed and must be recirculated continuously so as to cause a minimum of alteration in formulation from that intended. More than one granule size from a crumbling process is desirable to minimize the amount of under- and oversized granules to be recirculated.

If necessary, a binder specified by the Ministry may be used, substituting for a portion of wheat middlings or whey.

To make a pellet or granule of satisfactory durability, it is necessary that a major part of the oil be sprayed on the feed following pelleting, crumbling, and screening and allowed a significant time for oil absorption prior to bagging so that seepage will not occur.

Bags shall contain not more than 10% oversize or undersize granules. The "fines" content (defined as particle size < 0.4 mm) shall not exceed 2% of the feed.

Bagging and loading: The fish feed will be shipped in bags or by bulk truck at the buyer's discretion. If not specified, shipment will be made in 25-kg bags. The pellets and granules are not to be bagged or loaded for bulk delivery until cooled to 3°C above ambient air temperature and the moisture content should never exceed 10%. For those pellets and granules that are to be shipped in bags, the bags must be impervious to oil seepage and nonslip. The net weight of feed in each bag shall be 25 kg.

Labeling: Each bag must be clearly labeled with the formula name (e.g., MNR-84S), pellet or granule size (e.g., 3 Gr or 4 Pt) and date processed (e.g., 82-07-01) in the exact format given. Glue-on stickers or stamps are the only acceptable methods of labeling. The label must be located less than 10 cm from the bottom or side so that the labels can be read when the bags are stacked. The letter size for the formula and feed size should be larger than 2 cm^2.

Delivery

Delivery will be required prior to the date designated in each order of the contract, within 14 days of manufacturing date. All deliveries of fish feed, under award made as a result of this invitation for bid, shall be made only by licenced common carriers or contractor-owned trucks.

All feed (bulk or bagged) shall be loaded on suitable trucks at the feed mill and delivered directly to the designated lateleries on the same trucks unless otherwise specified in the bidding schedule.

The contractors must arrange their shipment schedules to avoid deliveries on Saturdays, Sundays, and government holidays. Shipments shall be scheduled to arrive between 0800 and 1600 hours Monday through Friday. Feed trucks arriving after 1600 hours will not be unloaded until the following workday. Twenty-four hour advance notice of the delivery date is required.

Conditions for Award

(1) The Ministry reserves the right to inspect the manufacturers' plants and production facilities prior to releasing tender documents and will reject bids by manufacturers whose plant facilities do not satisfy the Ministry's requirements (Table 23).

(2) It will be assumed that the equipment described in the above sections is functional and operational at the time of request for the inspection; otherwise, the application will not be considered. The manufacturer must be able to demonstrate the capability to manufacture all or part of the feed in the contract to the satisfaction of the Ministry if requested to do so.

(3) Award will not necessarily be made on the basis of the lowest tender and will be made only to recognized feed manufacturers in Canada.

(4) The quantity specified herein is estimated; the Government, there-

Table 23. Checklist for fish manufacturing facilities.

	Manufacturer			Notes
	1	2	3	
Housekeeping				
Grinder				
Premixer				
Mixer				
Scales				
Steam supply				
Boiler pressure				
Trap and piping				
Pellet mill				
Production capacity				
Dies				
Cooler				
Crumbler				
Screener				
Screens				
Fat Sprayer				
Meter				
Storage tank				
Storage bins				
Ingredient				
Feed				
Ingredients supply				
Production records				
Personnel				
Quality-control laboratory				
Others				

fore, reserves the right to increase or decrease the quantity by as much as 20% without changes in the tendered price.

(5) The Ministry intends to award this contract to a single supplier. However, the Ministry reserves the right to award the contract to several suppliers if it is in the best interest of the Ministry to do so.

(6) The contractor shall submit two confirmations of order of a sufficient quantity of quality fish meal and fish oil to assure supply for the following periods: 1 June 1985 – 31 October 1985 and 1 November 1985 – 31 October 1986 by the following dates: 1 June 1985 and 1 September 1985 respectively.

(7) The formulae supplied for this contract are the property of the Ministry and confidential. However, permission may be granted upon written request to manufacture feeds for sale using these formulae.

Inspection and Quality Control

(1) The Ministry reserves the right to inspect the contractors' plant facilities, equipment, inventories, and invoices from their suppliers during the contract period. The contractor will supply or make available during inspections labels and representative samples of all ingredients used upon request by personnel authorized by the Ministry.

(2) Thirty days prior to manufacturing, the contractor must submit a certificate of analysis of fish meal and fish oil (Table 12), provided by an independent testing laboratory specifying the lot number, detailed sample description (e.g., herring or capelin meal), origin of the ingredients to be used in the formulae, and the date samples were received by the laboratory.

Each shipment of ingredients must be analyzed separately.

(3) The contractor shall notify the Fisheries Branch, Ministry of Natural Resources, at least 14 days in advance of the exact mixing dates and schedule to allow arranging for inspection of ingredients, manufacture, and finished products.

(4) The contractor shall keep representative samples of fish meal, fish oil, feather meal, and vitamin and mineral premixer used in each production run and of final products until 6 months after the end of the contract year and supply representative samples if requested by the Ministry.

(5) Quality, including the level of contaminants, of the ingredients and fish feed shall be solely the responsibility of the contractor who selects the ingredients and manufactures the fish feed.

(6) The contractor shall keep a record of the detailed formula of any other feed manufactured, using the same equipment, prior to the processing of the Ministry's fish feed.

(7) The contractor must follow the supplied formulae strictly and any modification or substitution must be authorized by the Ministry prior to manufacturing.

(8) It is the responsibility of the contractor to ensure that competent personnel are employed and that they are fully informed of these "manufacturing specifications."

Violation of Contract Conditions

(1) The Ministry reserves the right to cancel any contract with a contractor for the supply of fish feed if any of the terms or conditions of the contract are violated. The contractor will receive 7 days written notice.

(2) Prices are to remain firm for the period of the contract. In the event of any unavoidable price increase, the Ministry requires 30 days written notice to renegotiate or ask for a second tender for the product lines affected.

(3) The Ministry reserves the right to change formulae specifications as necessary during the life of the contract, on 30 days notice.

If the Ministry requires changes, the contractor may: (a) continue to supply, at the original price, the new product line(s), or (b) decline to supply the new formulae at the original price. In the second case, the Ministry may make alternate supply arrangements for the product line(s) affected as it sees fit; and the successful contractor will continue to supply the other product line(s) that are not changed at the original tendered prices. The contractor should, therefore, tender prices on an individual "product line" basis. Tendered prices must be submitted on the form supplied in the tender package for this purpose.

(4) The contractor shall compensate for any damages to the Ministry's fish caused by the feed not meeting the specifications, as in: (a) a shortage or excess of any or several of the specified components making up the diets; (b) contamination by foreign materials either in the ingredients or added during manufacture of the feed; or (c) poor quality feed or feedstuffs.

Compensation may be made by the Ministry returning the shipment in question without payment to the contractor and at the contractor's expense, or by financial compensation to the Ministry by negotiated agreement as to the cost of the damages.

(5) Failure of the Ministry to insist, in one or more instances, upon the performance by the contractor of any term or condition outlined in this document shall not be construed as a waiver of the future performance of

any such term or condition and the obligations of the contractor with respect to such future performance shall continue in full force and effect.

Storage of Feedstuffs

The storage of feed ingredients and diets is another important step in maintaining the quality of well-processed feeds. During storage, there are many ways that feed quality can be diminished, including shrinkage or waste, chemical deterioration, and infestation by insects or microorganisms. Each of these problems results not only in the physical loss of feed but also, and most important of all, a reduction in the nutritive value of the diets for fish.

The main concern of the nutritionist with respect to vitamins should be to ensure that the requirements are met and that an additional allowance is made for an adequate safety margin in the final product. This is due to the fact that despite the availability of adequate information on vitamin requirement levels, at least for a number of species, and most vitamins are supplemented in crystalline form, vitamin deficiency disorders still occur in practical fish culture. This is partly because of the lability of vitamins coupled with improper manufacturing, handling, and storage procedures associated with fish feed. Many vitamins are especially susceptible to destruction by oxidation in the presence of excessive moisture, heat, and pro-oxidant metals.

One of the most labile vitamins is ascorbic acid, a reducing agent and a component of the prolyl hydroxylase system. Consequently, retained levels of ascorbic acid in practical diets give some indication of manufacturing and storage conditions. Work at the University of Guelph, Guelph, Ontario, has demonstrated that destruction of ascorbic acid occurs not only at high temperatures but also when it is in solution in water, as in the case of mashed or moist pellets (Hilton et al. 1977). Because there is no foolproof method of ensuring a given level of ascorbic acid in diets, a stable, protected form of the vitamin is needed.

To avoid or minimize losses during storage, some precautionary steps can be taken to eliminate most of the problems that are caused by humidity, temperature, sunlight, and hygiene conditions of feed under storage. At worst, these conditions will accelerate infestation by insects and microorganisms and putrefaction of protein, and cause fats to become rancid. This is a much more serious problem for fish feed, which contains much higher levels of protein and fat, than other animal feeds. There is particular danger in tropical regions where all of these hazards are present to a greater extent than in temperate regions. Refrigeration and freezing of feeds, avoidance of direct sunlight, inclusion of antioxidants in feeds and fats, and reduction of moisture content of diets (10–15%) all help to reduce deterioration of feed. Periodic checks of ascorbic acid and peroxide levels may assist in detecting the deterioration of feeds at an early stage. Under no circumstances should poor quality feed be fed to fish. Loss of feed is much less costly than loss of fish!

Diet Evaluation

The value of a diet depends upon the levels of available nutrients (about 40) that have been shown to be needed by fish. The nutritive value of

the diet for the fish, however, is especially difficult to define because of the interactions occurring among the food-derived substrates after they have been absorbed from the digestive tract. Nevertheless, it is important to define the energy value of the diet as this is a determining factor in terms of the amount of feed consumed by the fish because they adjust their food intake to satisfy their needs for energy. Thus, the actual intake of nutrients is regulated by the available energy level of the diet and energy requirements of the fish.

The simplest measures of available energy and nutrient levels in a diet are the digestibility values because these values do not appear to be influenced by levels of feeding above the maintenance level or by other environmental factors. This simple measure does not, however, provide any indication of the interactions that can influence the proportion of the dietary intake that is retained by the fish and used for growth. The advantage of using apparent digestibility values to compare different feed ingredients is that these values are additive, i.e., if the formula of the diet and digestible energy and nutrient values of the ingredients are known, the digestible energy and nutrient values of the mixed diet can be calculated.

Figure 6 shows that the loss of feces from the diet is the primary reason for variation in the nutritional value of foods. Measurement of digestibility gives a good indication of the availability of energy and nutrients in ingredients, thus providing a rational basis upon which diets can be formulated to meet specified standards of available nutrient levels. Losses that occur after the digestion of food, such as losses through the gills or in the urine, depend upon the appropriateness of the nutrient balance, level of feeding, and physiological status of the fish. None of these factors are

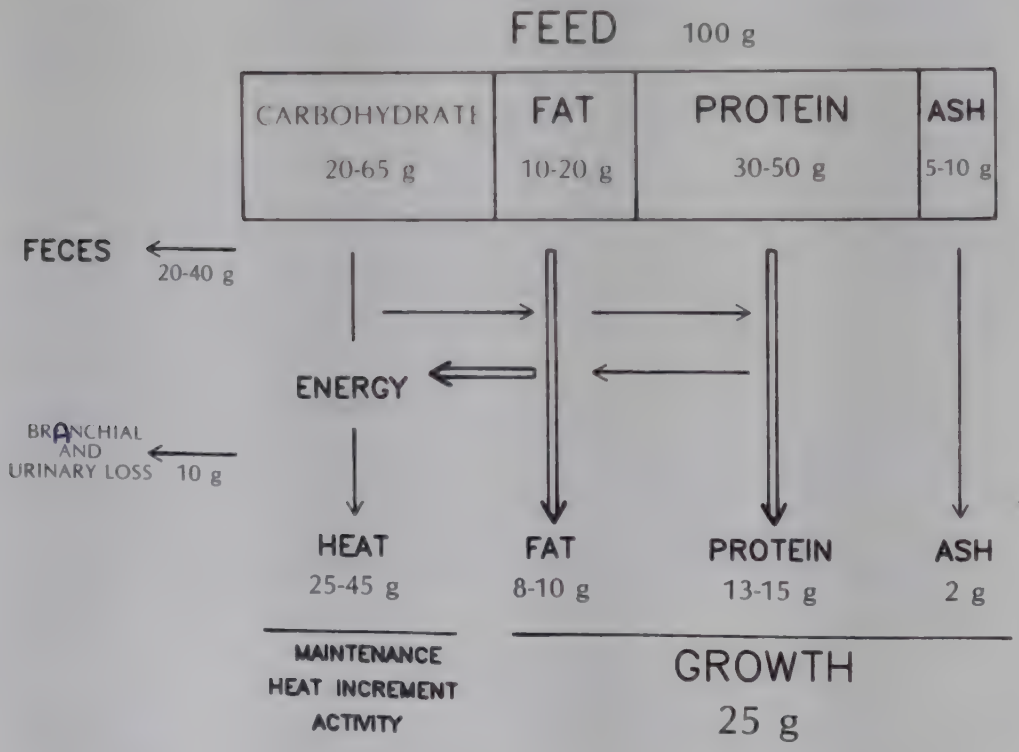


Fig. 6. Utilization of feed by rainbow trout.

directly attributed to the inherent characteristics of the ingredients included in the diet, but depend upon the skill of the nutritionist in formulating the feedstuffs into a balanced diet with respect to the animal's nutrient needs.

The effectiveness of diets formulated upon the basis of digestible energy and nutrients can be evaluated by observation of weight gain, feed efficiency, and body composition of fish receiving the diets under particular culture regimes. Only if the diets formulated in this way fail to support standard levels of productivity should the whole area of post-absorptive losses be examined. This will require measuring the nonfecal losses and energy dissipated as the heat increment of feeding. The preparation of a diet with an inadequate amino acid balance may be the cause of excessive nitrogen losses through the gills and in the urine. An inadequate balance of energy and protein, on the other hand, may be the cause of excessive energy lost as heat increment of feeding. Measuring these post-absorptive losses requires much more sophisticated facilities and more invasive techniques, which leads to stress for the fish, than measuring digestibility.

Feedstuffs that are believed to have potential for use in fish diets may be satisfactorily evaluated using the following scheme: (1) analyzing ingredients for their composition; (2) measuring digestibilities of feed ingredients; (3) formulating and reformulating balanced diets in combination with several ingredients or substituting with other ingredients; (4) observing the levels of productivity supported by such diets; (5) measuring feed intake and weight gain and calculating feed efficiency; and (6) calculating energy and nutrient retention efficiencies (NRE) by analyzing the fish carcasses. NRE can be calculated using the formula:

$$\text{NRE} = \frac{\text{nutrient gain in carcass}}{\text{nutrient intake} \times \text{digestibility coefficient of nutrient}}$$

Measurement of Feed Ingredient Digestibility

It is difficult to separate fish feces from the water and to avoid contamination of the feces by the uneaten feed. This problem has necessitated the use of very different approaches from those used to measure digestibility for mammals and birds. Nose (1960) collected samples of rectal contents by manually stripping the fish and gently squeezing out the fecal material from the rectum. Windell et al. (1978) obtained samples of rectal contents by applying suction to the anus or by dissecting the fish. The feces obtained by both of these techniques involved handling the fish and exposing them to considerable stress. Forced evacuation of their rectum would result in the addition of physiological fluid and intestinal epithelium to the rectal contents.

Smith (1971) confined the fish in metabolic chambers and collected the feces that were voided naturally into the water. Ogino et al. (1973) collected the feces by passing the effluent water from the fish tanks through a filtration column. Cho et al. (1975) used a settling column to separate the feces from the effluent water and Choubert et al. (1979) used a mechanically rotating screen to filter out fecal material.

The "Guelph system (CYAQ-2)" developed by Cho et al. (1975, 1982) to measure digestibilities by collecting fecal material in a settling column is shown in Fig. 7. There are three tanks in each unit, which all drain through a

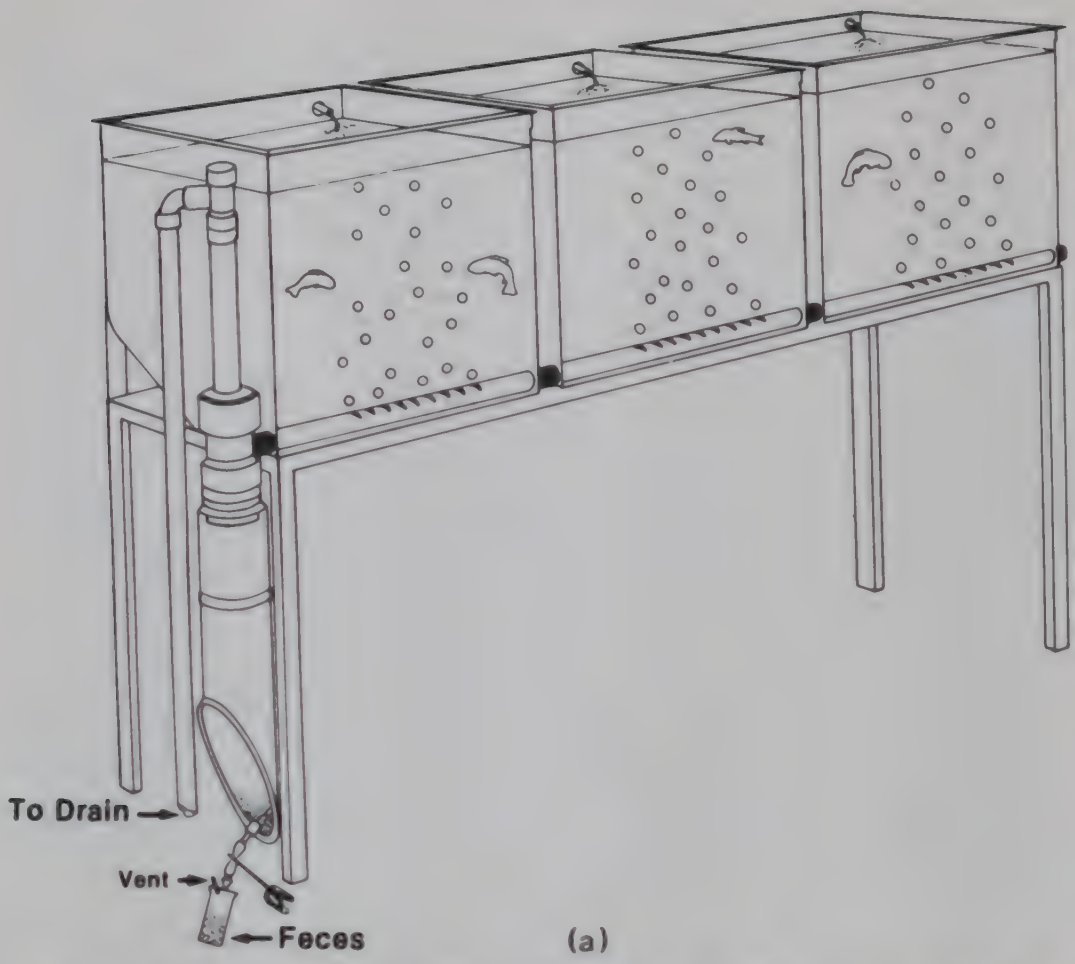




Fig. 7. (a) Setup for the collection of fecal material. (b) Collection of feces from the settling column using a large syringe.

common drainpipe, and a single standpipe placed over an acrylic settling column (10 cm diameter × 40 cm high). The base of the settling column can be surrounded by a cooling jacket to minimize degradation of the fecal material. The tanks each measure 55 cm × 40 cm × 35 cm and have a sloped bottom. Each tank is loaded with 5–6 kg of fish. The velocity of the water flow is adjusted to minimize settling of the feces in the drainpipe and maximize recovery of the feces in the settling column while maintaining appropriate levels of dissolved oxygen and ammonia in the water. Under normal operation, it has been observed that larger feces particles are trapped in the settling column within 2 min of being voided by the fish.

The fish are accustomed to both the tanks and the dietary regime for at least 3 days before collection of feces begins. The fish are fed three meals daily between 0900 and 1600 hours, the diets being offered only as long as the fish are actively feeding to avoid wastage. The fish are fed normally throughout the day. One hour after the last meal, the drainpipe and the settling column are brushed out to remove feed residues and feces from the system. One-third of the water in the tanks is drained out to ensure that the cleaning procedure is complete. At 0830 hours the following day, the settled feces and surrounding water are gently withdrawn from the base of the settling column into a centrifuge bottle. These feces are free of uneaten feed particles and are considered to be a representative sample of the feces produced throughout the 24-hour period. Immediately after collection of the feces, the fish are fed again as normal, allowing repeated sampling over 6–9 days (Fig. 7; Table 24).

The feces are centrifuged at 10 000 × *g* for 20 min and the supernatant discarded. The feces are then freeze-dried and ground for determination of chromic oxide concentration, nutrient analysis (dry matter, nitrogen, and fat), and gross energy.

A series of eight such units allows the determination of digestibility coefficients for up to seven ingredients at any one time; one unit being devoted to the reference diet. This enables one to examine the influence of fish size, meal size, and water temperature on the digestibility of feeds. The fish are under the culture regime normally applied to other nutritional experiments, thus allowing the application of laboratory standards in digestibility determinations based on levels of feed consumption and weight gains of the fish.

The advantages of the "Guelph system" are that it allows the fish to feed normally, there is no need to handle the fish, it allows repeated determinations, and evaluation of different diets can be carried out at the same time as observations of apparent digestibility, growth rate, and carcass analysis are made. One criticism of this method is that soluble material would be lost from the feces due to leaching. The close agreement between digestibilities of dry matter, crude protein, and lipids obtained by intestinal dissection and collecting rectal contents by suction with results obtained using the settling column, however, indicates that leaching is not an important source of error. The major affect of leaching is the "breakup" of feces particles (handling loss) that results from physical handling, which must be avoided.

Very few potentially useful feed ingredients can be fed voluntarily as the sole component of a diet as it is always necessary to combine a mixture of feed components in formulating a diet. Thus, determination of the digestibility of a feed requires comparing the digestibilities of a reference

Table 24. Reference and test diets for digestibility studies.

	Reference diet	Test diet
Reference diet (%)	100	70
with 1% chromic oxide	0	30
Test ingredients (%)		

Procedure for feces collection using the "Guelph system (CYAQ-2)":

(1) Prepare reference and experimental diets: (a) reference diet with 1% chromic oxide (nutritionally well-balanced and supports good growth); (b) experimental diet, which consists of 30% test ingredient and 70% reference diet.

(2) Weigh feed and fish in each tank (approximately 1 kg/10 L water) and set water temperature as required.

(3) Feed diets for at least 3 days without feces collection.

(4) Adjust flow rate to set the water velocity in the drainpipe, which maximizes the amount of feces collected in the settling column.

(5) Brush inside of tank; drain system, standpipe and settling column; and flush out almost half of the water 1 hour after the last feeding of the day (Note: no feeding until the next feces collection).

(6) In the morning, before the first feeding, withdraw feces very gently from the bottom of the settling column using a large hypodermic syringe (~50 mL) or centrifuge bottle with stopper and needle vent (Fig. 7). There must be absolutely no disturbance of the feces while collecting to avoid handling loss by "break-up" and, hence, leaching losses.

(7) Centrifuge feces to remove excess water and freeze.

(8) Feed fish according to the normal daily feeding schedule for growth requirement after morning collection of feces. (Note: feeding level should be near satiety to minimize the difference between apparent and true digestibilities. Feces collected under conditions of abnormally low gain and feed intake (not "amount fed") cannot be used for the determination of apparent digestibility coefficients of feedstuffs because the proportion of endogenous excretion to undigested nutrients is too high.)

(9) Daily brushing and flushing of the system 1 hour after the last daily feeding is repeated (steps 5 – 9) until enough feces is collected — approximately more than 1 g feces/kg body weight·day^{-1} (lyophilized weight) can be collected.

(10) Measure feed intake and live weight gain during experimental period. If negative or unacceptably low gain and feed intake, discard feces sample.

(11) Analyze diet and feces for nutrients and indicator (three consecutive, 2 – 3 days feces collections each being pooled).

(12) Calculate apparent digestibility coefficients (ADC) of components of test ingredient using the following formulae:

$$\text{ADC of reference or experimental diet} = 1 - (F/D \times Dcr/Fcr)$$

$$\text{ADC of test ingredient} = (\text{ADC of experimental diet} - 0.7\ \text{ADC of reference diet})/0.3$$

Where: F = % nutrient in feces,
D = % nutrient in diet,
Fcr = % chromic oxide in feces, and
Dcr = % chromic oxide in diet.

and a test diet; the test diet being a mixture of the reference diet, test ingredient, and chromic oxide, which is used as a digestion indicator (Table 24). Inclusion of 1% chromic oxide in the reference diet allows the digestibility coefficients of the nutrients in the test diets to be calculated from measurements of the nutrient to indicator ratios in the diet and feces. Edin (1918) introduced the use of chromic oxide as a digestion indicator to obviate the need to quantitate dietary intake and feces output. Austreng (1978) confirmed the suitability of this procedure for measuring digestibilities of fish. Once these coefficients have been calculated for the reference and test diets, the corresponding digestibility coefficients can be calculated for the nutrients in the ingredient being tested.

The use of a reference diet assumes that there are no interactions between the components of the diet during digestion. As well, adoption of this

procedure allows the preparation of an adequately balanced diet with which to test the susceptibility of the feedstuff to digestion. In determinations using reference and substituted diets, measurement of feed intake and growth rate allowed confirmation of the nutritional adequacy of the experimental diets.

Feeding Practices

Feeding Strategy and Water Temperature

The main factors influencing the intake of feed that is organoleptically acceptable to fish are water temperature and energy content of the diet. Water temperature influences metabolic rate and energy expenditure. Because most species of fish consume food to satisfy their energy requirements, the energy content of the diet determines the amount of feed consumed. As shown in Fig. 8, the growth rate of rainbow trout is controlled by water temperature and the total requirement for feed is proportional to the rate of live weight gain.

Feeding of fry must start as soon as the fish absorb their yolk sac and begin to swim up. At this stage, the fry must be fed at least once every hour during the normal light period and may be slightly overfed as long as wasted feed is removed regularly. Water temperature should be kept high enough to ensure that swim-up fry start to eat at the earliest possible moment. Fish may never regain lost growth at this early stage of development so it is important to maintain maximum feed intake and ensure adequate water temperature for the normal metabolic processes.

Fish must be fed properly sized granules or pellets. When necessary, the feed should be screened to remove fines and granules that are too small or too large. Fish must be fed moderately and not overfed. Because of increased nutrient density in today's diets, less feed is now recommended, particularly for starter diets. This can be achieved by reducing the frequency of feeding (or the amount of feed per feeding on some occasions). Many feeding guides are descendants of the meat-meal diets of the past or "guesstimates" and, therefore, caution should be exercised in applying them to the diets available today. In many cases, feeding slightly below satiety by experienced culturists is preferred to the feeding guides that are supplied so readily by various sources.

During the summer or hot season, when the water temperature is high, the feeding time, feeding frequency, and amount of feed given at each feeding should be adjusted according to the level of dissolved oxygen in the water. Estimates based solely upon biomass and feeding charts can be misleading and are a potential source of problems. Giving less feed at each feeding while increasing the frequency of feedings can help prevent any low dissolved oxygen problems. Wasted feed can further cause gill damage and support fungal and bacterial growth, all of which can lead to disease. Feeding, when done by hand, should occupy a considerable portion of the daily routine, particularly for young fish. To ensure that all feed is eaten, only small amounts should be broadcasted at any one time.

At low water temperatures, fingerlings and larger fish may not require frequent feeding for growth (e.g., once a day may be sufficient) as long as wasting of feed is avoided. At these low water temperatures, no feed for

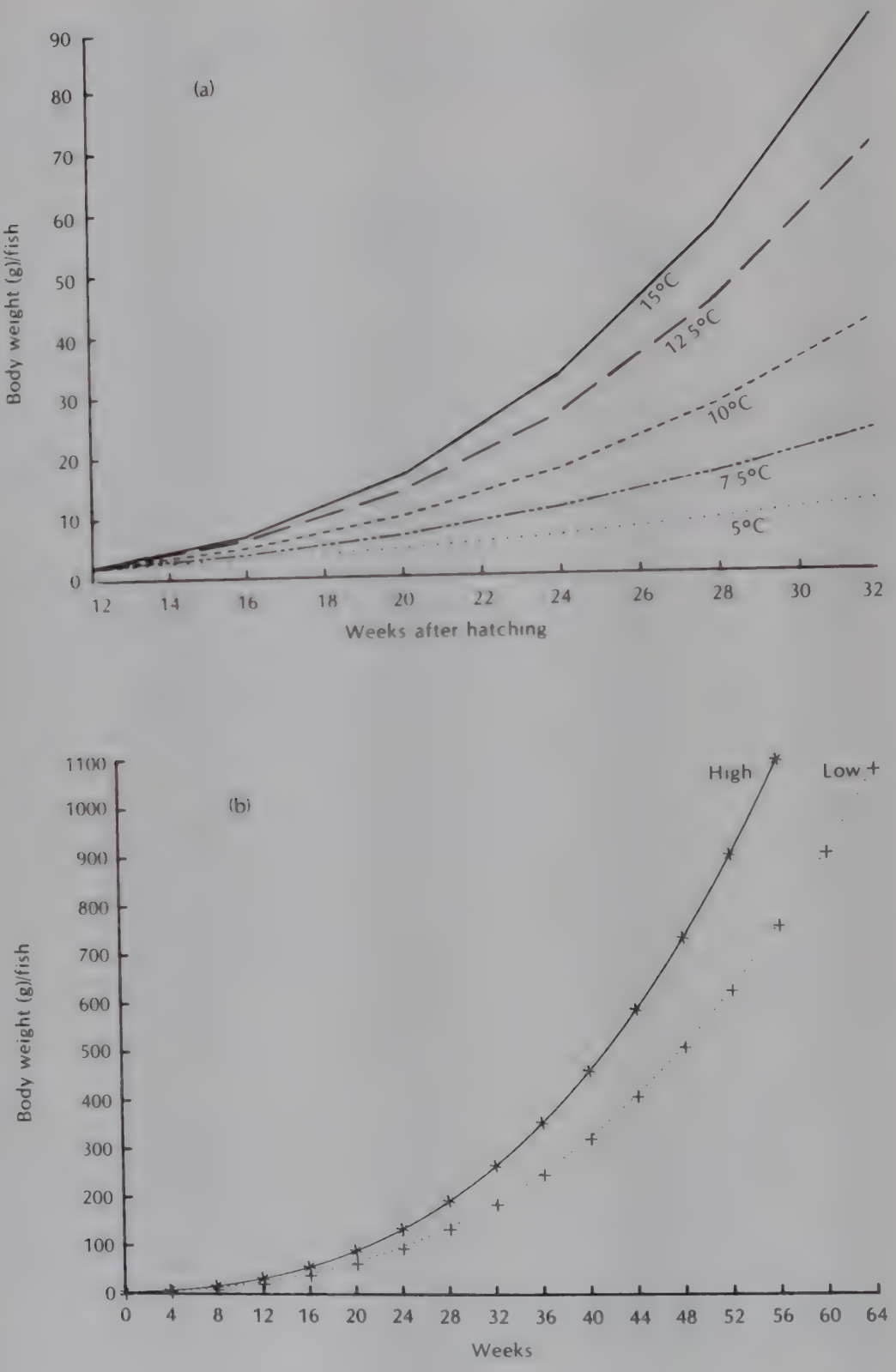


Fig. 8. (a) Growth of rainbow trout at various water temperatures. (b) Predicted growth (high and low) of sea bass. (Based on data from Renee Chou, Aquaculture Unit, Primary Production Department, Singapore.)

several days will not harm the fish; in fact, the energy cost for utilizing consumed feed may aggravate the situation at such low temperatures. If a group of fish is not feeding actively, it should be given immediate attention as a lack of appetite is an early sign of stress and disease. The sizes of feed, screen openings, and suggested frequency of feeding are shown in Table 22. However, feeding frequency should be adjusted as required under local conditions.

Dietary Energy Level and Total Feed Requirements

Because feed intake of fish is controlled mainly by body weight and expected gain, water temperature, and energy content of the feed, the first step in estimating feed requirements on a rational basis is to predict the expected live weight gain in a given time period and under the given water temperature regime. Iwama and Tautz (1981) reported a growth model based on the one-third power of body weight, which fits the growth curves of salmonids much better than the previously reported exponential model (specific growth rate). Therefore, total feed intake was calculated on the basis of predicted gain by estimating the temperature constant (TC) developed from the growth model of the one-third power and the dietary digestible energy requirement of 15 MJ/kg live weight gain measured by Cho (1982).

The procedure for predicting body weight and calculating live weight gain and feed intake is summarized in Table 25. The temperature constant of a fish culture operation must be estimated from previous years' records on the assumption that factors such as genetic strain, dietary regime, water temperature, and other management practices are basically unchanged. The digestible energy (DE) in a diet can be calculated from the information on diet formula contained in Table 16. Otherwise, the DE content of a diet, based upon levels of protein and fat, can be estimated as indicated in Table 25, (5c). Sample calculations are presented in Table 26 and, to facilitate computation, the values of the one-third power of body weight between 1 and 499 g fish are listed in Table 27. Once the live weight gain and total feed intake are estimated for the next 4-week period, the feed is distributed on a weekly basis according to the growth rate (Table 26, (5)). However, this projected feed intake is only for use as a guide and under a sensible system of feeding the fish will ultimately determine the quantity consumed.

Mechanical (Automatic) and Pendulum (Demand) Feeders

Feeding fish is the most important daily activity at fish culture stations. It utilizes a considerable proportion of staff time. Mechanical means of partially replacing hand feeding by fish culturists include: (1) mechanical feeders, which are activated by a timer, and (2) pendulum feeders, which are activated by the fish. The latter is also known as a demand feeder. These mechanical feeders can be useful to free up some of the time formerly devoted to hand feeding. The feeder, on the other hand, must be calibrated and adjusted regularly as the amount of feed dispensed also depends on the type and size of feed. A misconception about the "demand" feeder is that it

Table 25. Prediction of growth and expected feed intake.

1. Temperature constant (TC)
   Initial body weight = Wo (g/fish)
   Final body weight = Wt (g/fish)
   (This value is calculated with previous years' growth data and is influenced by genetics, diet, water quality, and other management practices. It must be checked and recalculated regularly, therefore, for different lots of fish.)

$$TC = \frac{\Sigma\ (\text{temp.}(^{\circ}C) \times \text{days})}{Wt^{1/3} - Wo^{1/3}}$$

2. Expected live body weight (Wt)[a]

$$Wt\ (g) = \left[Wo^{1/3} + \frac{\Sigma\ (\text{temp.}(^{\circ}C) \times \text{days})}{TC}\right]^3$$

3. Expected total live weight gain (TGN)
   TGN (g) = (Wt − Wo) × no. of fish

4. Expected total feed intake (TFI)

$$TFI\ (kg) = \frac{0.015 \times TGN\ (g)}{\text{MJ DE per kg feed}}$$

5. Estimating digestible energy of diet (DE)
   (Assume nutrients are balanced based on digestible energy)
   Options:
   (a) Obtain from your feed formula or manufacturer
   (b) Calculate DE value of your diet using information from Table 16 and
      37 MJ per kg fish and plant oils
      33 MJ per kg animal fats
   (c) Approximate DE values of your diet with
      40% crude protein/10% fat diet = 14 MJ/kg feed
      40% crude protein/15% fat diet = 16 MJ/kg feed
      45% crude protein/10% fat diet = 15 MJ/kg feed
      45% crude protein/15% fat diet = 17 MJ/kg feed

[a] Modified from Iwama and Tautz (1981).
Note: See Tables 26 and 27 for example calculations.

never overfeeds; however, it is a very useful tool for replacing hand feeding as shown by the data on feed intake and growth presented in Table 28. The selection of different types of mechanical feeders depends on the design of the pond and raceway. For larger systems, the feeder that can broadcast over a wider area, such as a pneumatic feeder, may be preferable. Overall, mechanical feeders only replace manual feeding, never feeding strategy. A type of pendulum feeder is shown in Fig. 9.

Nutrition of Broodstock and Larvae — Status Quo

Effect of Nutritional Quality of Broodstock Diets on Egg Development

The ever-growing demand of fish breeders for artificially produced seed requires, ideally, a year-round (rather than seasonal) supply of fertile eggs of high quality that give as high survival and growth rates as those occurring naturally. The former requirement — year-round production — has been achieved with some species by adjustment of the endocrine balance through variation in the photoperiod or temperature regime.

Nutrition is known to have a considerable effect upon gonadal growth

Table 26. Growth prediction and feed intake estimation.

1. Calculate temperature constant (TC)
   Example: Wo = ave. 10 g/fish (1980.04.01) Total 112 days
   Wt = ave. 65 g/fish (1980.07.21) growing period
   Water temp. 11°C for 49 days
   13°C for 63 days

$$TC = \frac{(11 \times 49) + (13 \times 63)}{65^{1/3} - 10^{1/3}} = \frac{539 + 819}{4.021 - 2.154} = 727$$

   (To calculate $Wt^{1/3}$ and $Wo^{1/3}$ refer to Table 25.)

2. Calculate expected live body weight (Wt) of 1981 production
   Example: Wo = ave. 18 g/fish (1981.05.01)
   Expected water temp. 11°C for 10 days
   12°C for 18 days
   Temperature constant (TC) in 1980 = 727
   Estimate body weight (Wt) on 1981.05.29

$$Wt = \left[18^{1/3} + \frac{(11 \times 10) + (12 \times 18)}{727}\right]^3$$

$$= [2.621 + (110 + 216)/727]^3 = 3.07^3 = 29 \text{ g/fish}$$

3. Estimate total live body weight gain (TGN) of 10 000 fish
   $TGN = (29 - 18) \times 10\,000 = 110\,000$ g

4. Estimate total feed intake (TFI) of 10 000 fish for 4 weeks
   Example: Present diet contains 40% crude protein and 15% fat and use "option 5c" from Table 25.
   Estimated digestible energy of diet = 16 MJ/kg feed

$$TFI = \frac{0.015 \times 110\,000}{16} = 103.1 \text{ kg feed in 28 days}$$

5. Calculate weekly feed intake (WFI) for coming 4 weeks
   Example: TFI = 103 kg
   WFI = 103 kg × 0.2 = 20.6 kg feed in 1st week
   WFI = 103 kg × 0.23 = 23.7 kg feed in 2nd week
   WFI = 103 kg × 0.27 = 27.8 kg feed in 3rd week
   WFI = 103 kg × 0.3 = 30.9 kg feed in 4th week

and fecundity, although the few published papers on the subject have given inconsistent results. Consequently, precise information on the nutritional requirements for gonadal maturation in broodstock is lacking.

Recently, Watanabe et al. (1984a,b,c) in Japan have carried out nutritional experiments on brookstock of both rainbow trout and red sea bream. In an experiment involving rainbow trout, fingerlings (3.5 g live weight) were grown at natural water temperatures (5–20°C) for 3 years and given either a commercial diet (43–47% crude protein) or one of three experimental diets (two of these were low protein/high energy diets, 33–35% crude protein and 390 kcal/100 g, and the other contained no trace element supplement).

The average gain over the experimental period was 1.5 kg, except for the trout given the diet lacking a mineral supplement (these gained 1 kg on average). During this time, the fish spawned twice but the quality of eggs was examined only after the second spawning as eggs produced during the first spawning are usually low in quality. For the second spawning, there were no differences in egg production per female (approximately 3000), egg diameter (average egg diameter 5.2 mm), the proportion of eggs reaching the eyed stage (90%), and the proportion hatching (87%) between

Table 27. Values of the one-third power of body weight.

Body weight (g)/fish	0	1	2	3	4	5	6	7	8	9
0	0.000	1.000	1.260	1.442	1.587	1.710	1.817	1.913	2.000	2.080
10	2.154	2.224	2.289	2.315	2.410	2.466	2.520	2.571	2.621	2.668
20	2.714	2.759	2.802	2.844	2.884	2.924	2.962	3.000	3.067	3.072
30	3.107	3.141	3.175	3.208	3.240	3.271	3.302	3.332	3.362	3.391
40	3.420	3.448	3.476	3.503	3.530	3.557	3.583	3.609	3.634	3.659
50	3.684	3.708	3.733	3.756	3.780	3.803	3.826	3.849	3.871	3.893
60	3.915	3.936	3.958	3.979	4.000	4.021	4.041	4.062	4.082	4.102
70	4.121	4.141	4.160	4.179	4.190	4.217	4.236	4.254	4.273	4.291
80	4.309	4.327	4.344	4.362	4.380	4.397	4.414	4.431	4.448	4.465
90	4.481	4.498	4.514	4.531	4.547	4.563	4.579	4.595	4.610	4.626
100	4.642	4.657	4.672	4.688	4.703	4.718	4.733	4.747	4.762	4.777
110	4.791	4.806	4.820	4.835	4.849	4.863	4.877	4.891	4.905	4.919
120	4.932	4.946	4.960	4.973	4.987	5.000	5.013	5.027	5.040	5.053
130	5.066	5.079	5.092	5.104	5.117	5.130	5.143	5.155	5.168	5.180
140	5.192	5.205	5.217	5.229	5.241	5.254	5.266	5.278	5.290	5.301
150	5.313	5.325	5.337	5.348	5.360	5.372	5.383	5.395	5.406	5.418
160	5.429	5.440	5.451	5.463	5.474	5.485	5.496	5.507	5.518	5.529
170	5.540	5.550	5.561	5.572	5.583	5.593	5.604	5.615	5.625	5.636
180	5.646	5.657	5.667	5.677	5.688	5.698	5.708	5.718	5.729	5.739
190	5.749	5.759	5.769	5.779	5.789	5.799	5.809	5.819	5.828	5.838
200	5.848	5.858	5.867	5.877	5.887	5.896	5.906	5.915	5.925	5.934
210	5.944	5.953	5.963	5.972	5.981	5.991	6.000	6.009	6.018	6.028
220	6.037	6.046	6.055	6.064	6.073	6.082	6.091	6.100	6.109	6.118
230	6.127	6.136	6.145	6.153	6.162	6.171	6.180	6.188	6.197	6.206
240	6.214	6.223	6.232	6.240	6.249	6.257	6.266	6.274	6.283	6.291
250	6.300	6.308	6.316	6.325	6.333	6.341	6.350	6.358	6.366	6.374
260	6.383	6.391	6.399	6.407	6.415	6.423	6.431	6.439	6.447	6.455
270	6.463	6.471	6.479	6.487	6.495	6.503	6.511	6.519	6.527	6.534
280	6.542	6.550	6.558	6.565	6.573	6.581	6.589	6.596	6.604	6.611
290	6.619	6.627	6.634	6.642	6.649	6.657	6.664	6.672	6.679	6.607
300	6.694	6.702	6.709	6.717	6.724	6.731	6.739	6.746	6.753	6.761
310	6.768	6.775	6.782	6.790	6.797	6.804	6.811	6.818	6.826	6.833
320	6.840	6.847	6.854	6.861	6.868	6.875	6.882	6.889	6.896	6.903
330	6.910	6.917	6.924	6.931	6.938	6.945	6.952	6.959	6.966	6.973
340	6.980	6.986	6.993	7.000	7.007	7.014	7.020	7.027	7.034	7.041
350	7.047	7.054	7.061	7.067	7.074	7.081	7.087	7.094	7.101	7.107
360	7.114	7.120	7.127	7.133	7.140	7.147	7.153	7.160	7.166	7.173
370	7.179	7.186	7.192	7.198	7.205	7.211	7.218	7.224	7.230	7.237
380	7.243	7.250	7.256	7.262	7.268	7.275	7.281	7.287	7.294	7.300
390	7.306	7.312	7.319	7.325	7.331	7.337	7.343	7.350	7.356	7.362
400	7.368	7.374	7.380	7.386	7.393	7.399	7.405	7.411	7.417	7.423
410	7.429	7.435	7.441	7.447	7.453	7.459	7.465	7.471	7.477	7.483
420	7.489	7.495	7.501	7.507	7.513	7.518	7.524	7.530	7.536	7.542
430	7.548	7.554	7.560	7.565	7.571	7.577	7.583	7.589	7.594	7.600
440	7.606	7.612	7.617	7.623	7.629	7.635	7.640	7.646	7.652	7.657
450	7.663	7.669	7.674	7.680	7.686	7.691	7.697	7.703	7.708	7.714
460	7.719	7.725	7.731	7.736	7.742	7.747	7.753	7.758	7.764	7.769
470	7.775	7.780	7.786	7.791	7.797	7.802	7.808	7.813	7.819	7.824
480	7.830	7.835	7.841	7.846	7.851	7.857	7.862	7.868	7.873	7.878
490	7.884	7.889	7,894	7.900	7.905	7.910	7.916	7.921	7.926	7.932

treatments other than for trout given the diet lacking supplementary minerals. The respective values for that group were 2000 eggs/female, 5.1 mm egg diameter, 3.7% of eggs reaching the eyed stage, and 0.4% hatching.

These results demonstrate that a diet containing a lower protein content

Table 28. Performance of rainbow trout fed by hand and using a pendulum (demand) feeder.

Experimental period	Feeding methods	Live body weight (kg/100 fish)	Total feed intake (kg/100 fish)	Feed : gain ratio
After				
4 weeks	Hand	8.9	2.2	0.9
	Pendulum	8.6	2.3	1.0
8 weeks	Hand	12.7	6.9	1.2
	Pendulum	12.1	6.8	1.3
12 weeks	Hand	15.8	10.9	1.3
	Pendulum	15.3	10.7	1.2
16 weeks	Hand	17.9	14.0	1.5
	Pendulum	16.9	13.3	1.6

Notes: Initial body weight = 6.5 kg/100 fish ± 0.3; 60 fish per tank × 3 replications; water temperature = 15°C (flow-thru system); pendulum feeders donated by EWOS A/S, Sweden.

than that normally employed but with a high energy level is as effective for both fingerlings and broodstock of rainbow trout as the more conventional diets with high protein content. In contrast, a trace metal supplement added to the diet has been shown to be indispensable for reproduction of rainbow trout. Of the minerals analyzed, the most striking change was in manganese concentration, which fell from 4.1±0.7 μg/g eggs in females given a commercial diet to 1.6±0.1 μg/g in females given the experimental diet lacking a trace metal supplement.

In a second experiment with rainbow trout, broodstock were given experimental diets for only 3 months prior to spawning. Four of the treatments were used to examine further the effects of protein and energy balance on egg quantity and quality. It was confirmed that broodstock given a diet containing 36% crude protein and 18% lipid performed as well as those given a diet with 46% crude protein and 15% lipid. It was also shown that beef tallow used at a level of 7% as an energy source had no adverse effect on the reproduction of rainbow trout.

As anticipated, the use of partially defined diets deficient in essential fatty acids (EFA) led to the lowest values for total egg production, percentage of eyed eggs produced, and total hatch. It is of particular interest to note that addition of linoleic acid, 18:2(n-6), to the EFA deficient broodstock diet led to marked improvement in percentage of fertilization, percentage of eyed eggs, and total hatch compared with broodstock given diets lacking essential fatty acids. Linoleic acid is known to be inferior to linolenic acid, 18:3(n-3), as an EFA for rainbow trout fingerlings but the situation seems quite different with broodstock.

In this context, it is of considerable interest to note that rainbow trout have been grown through a generation using a semipurified diet containing 1% linolenic acid as the sole dietary EFA. The eggs produced, however, contained a small amount of arachidonic acid, 20:4(n-6). This demonstrates that the trout broodstock had tenaciously retained the small amounts of (n-6) fatty acids present in incompletely extracted diet ingredients such as casein, dextrin, and gelatin. It is possible, therefore, that there is a small but absolute requirement for (n-6) fatty acids in rainbow trout.

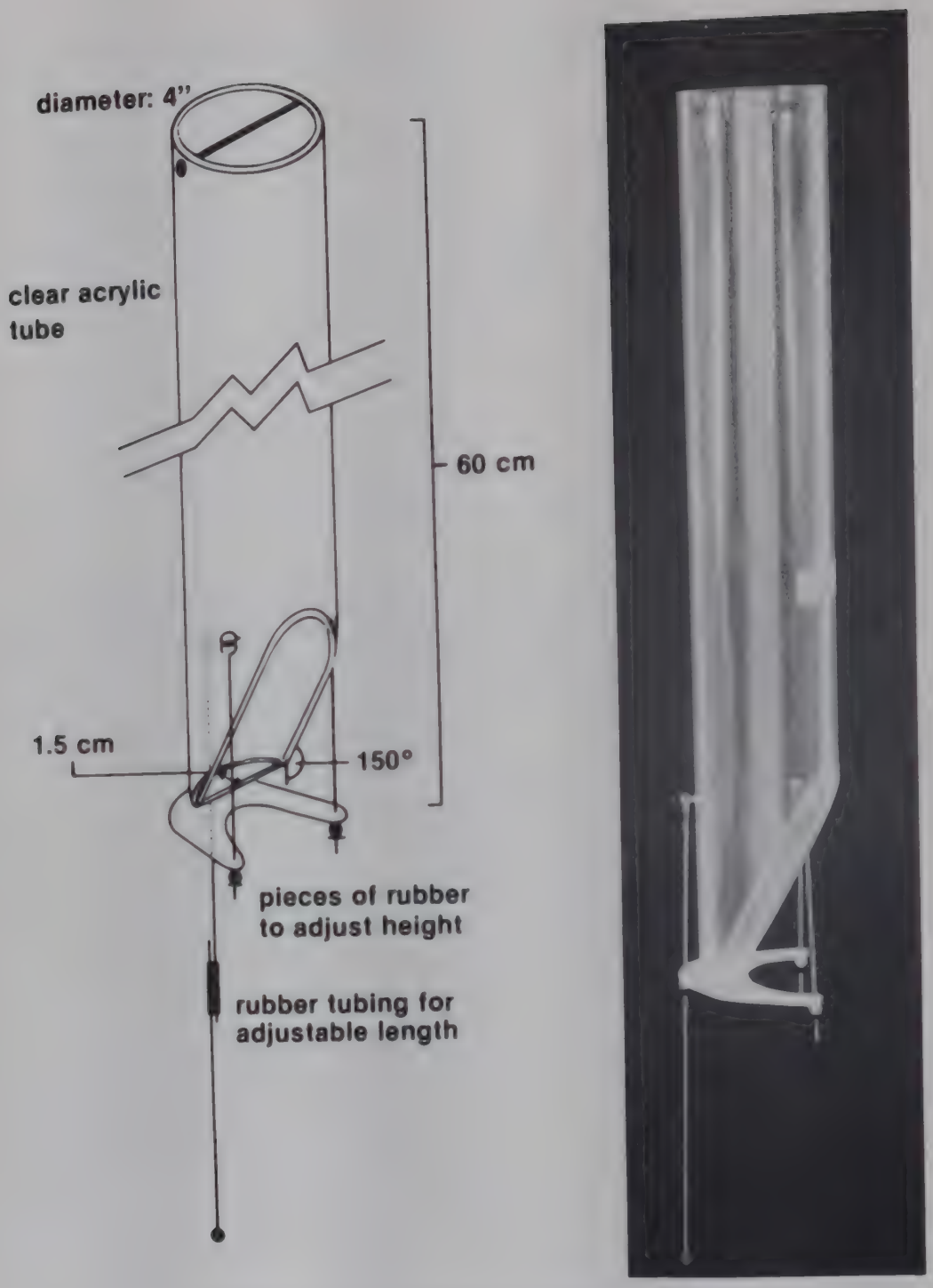


Fig. 9. Schematic diagram and photo of the CYAQ-7 pendulum fish feeder.

In red sea bream, it is well known that acceptable diets are actively eaten by the broodstock even during spawning and that feeding broodstock with krill, *Mysis*, shrimp, and crab wastes results in pigmentation of the eggs produced within a matter of hours. This suggests that the nutritional value of the diet given to broodstock shortly before spawning may affect the results of spawning. It also indicates that pigments and other fat-soluble materials

in diets are easily incorporated into eggs and the quality of eggs, therefore, may be improved by feeding broodstock with some fat-soluble nutrients such as essential fatty acids and vitamins.

This has been studied by Watanabe et al. (1984a,b,c) using broodstock that had been reared on diets (Table 29) containing either 55% protein (from fish meal) and 10% lipid (3% cuttlefish oil and the remainder from fish meal) or 45% crude protein (a mixture of fish meal and cuttlefish meal) and 10% lipid (3% cuttlefish oil and the remainder from the fish meals) until shortly before spawning. At this point, some of the broodstock were transferred to diets containing one or the other of carotenoid pigments (β-carotene 0.1%, canthaxanthin 0.3%), krill oil extract (9%; containing astaxanthin mono- and di-esters), or corn oil (at a level of 10% and replacing cuttlefish oil); some were given frozen raw krill; and others maintained the original diet.

Supplementation of diets with β-carotene and canthaxanthin or krill oil extract led to a slight decrease in the total number of eggs produced, but the percentage of buoyant eggs increased from 49.1% to 56.4% and 69.6%, respectively, in the fish given diets supplemented with pigments. Feeding frozen raw krill led to marked improvements in both the total number of eggs produced and percentage of buoyant eggs. The use of large amounts (10%) of corn oil in the diet, however, resulted in a marked reduction in the proportion of buoyant eggs produced.

Abnormalities in the number of oil globules in the eggs (percentage of eggs with more than two oil globules) were effectively reduced by the use of pigments, krill oil extract, or frozen raw krill. The effect was most marked in the latter treatment in which the percentage of abnormalities was 8.1% compared with a control value of 77.5%. In eggs from broodstock fed the diet containing corn oil, abnormalities in the number of oil globules increased to 94%.

The rate of hatching (83.1%) was not improved through the addition of

Table 29. Composition (%) of stock experimental diets used for red sea bream broodstock and experimental diets given just before spawning.

	Stock diets		Diets used just before spawning			
Fish meal	82	34	82	74.5	—	67
Cuttlefish meal	0	31	0	0	—	0
α-starch	7	15	7	9	—	15
Mineral mix	5	5	5	4.5	—	5
Vitamin mix	2	2	2	1.8	—	2
Choline chloride	1	1	1	0.9	—	1
Cuttlefish oil	3	3	3	0	—	0
Cellulose	0	9	0	0	—	0
Oil extracts from krill	0	0	0	9	—	0
Corn oil	0	0	0	0	—	10
β-carotene	0	0	0.1	0	—	0
Canthaxanthin	0	0	0.3	0	—	0
Frozen raw krill					100	
Nutrient content calculated						
Crude protein	55	45	55	51	—	45
Lipid	10	10	10	17	—	16
Nutrient content measured						
Crude protein	52.1	43.6	53.0	50.1	—	45.7
Lipid	9.5	9.0	9.9	14.9	—	16.6

β-carotene and canthaxanthin (77.4%) or krill oil extract (67.5%), but abnormalities in the number and position of oil globules in the hatched larvae were reduced to very low levels. Consequently, the proportion of normal larvae obtained increased from 51.6% in the control diet to 74.3% (β-carotene and canthaxanthin supplemented diet) and 88.2% (krill oil extract) with these treatments. The value obtained for those broodstock given frozen raw krill (91.2%) was even more striking. In contrast, in the broodstock fed the diet containing 10% corn oil, only 24% of normal larvae were produced.

Thus, the nutritional quality of diets given to broodstock of species like red sea bream, which can accept diets actively even during spawning, is seen to be important and may affect reproduction and egg quality. Frozen raw krill was especially suitable as a food source for red sea bream at this time. The superior results (over krill oil extract and pigments) obtained suggest that other factors, in addition to pigments, contribute to its value — perhaps quality and ease of digestion of protein.

The deleterious effect of diets containing corn oil at a level of 10% may partly reflect the accumulation of linoleic acid (18:2(n-6)) in the eggs and the inability of red sea bream to convert it to 20:4(n-6), arachidonic acid. It cannot be inferred that small quantities of the latter fatty acid are not useful or even desirable in the diet of red sea bream broodstock. The results indicate, however, a need for careful balancing of essential fatty acids in broodstock diets.

Nutritional Quality of Natural Food for Finfish Larvae

The feeding regime used most extensively in the production of larval fish of various species in Japan is shown in Fig. 10. Rotifers are the most important live food; in fact, mass production of *Brachionus* has made fish larvae production possible. Newly hatched larvae of body length 2.3 mm are given rotifers as the starting diet and this is continued for about 30 days. When the fish reach 7 mm or more in body length, marine copepods such as *Tigriopus*, *Acartia*, *Oithona*, and *Paracalanus* (or, in their absence, *Moina* and *Daphnia* of freshwater origin) are fed together with rotifers because rotifers are slightly small for larvae of 7 mm length. Brine shrimp, *Artemia salina*, are frequently used as a food for larvae of marine fish when there is a shortage of marine copepods. Larvae larger than 10–11 mm are fed on minced fish, shellfish, and shrimps or on an artificial diet. Larval stages are considered complete when juveniles attain a total body length of 30–50 mm.

Problems have been encountered over the nutritional quality of *Brachionus* and these were related to the food organisms used in its culture. When grown on a marine *Chlorella*, rotifers of high nutritional value were obtained, but when baker's yeast, *Saccharomyces cerevisiae*, or a freshwater *Chlorella* were used, the *Brachionus* produced were of variable and often inadequate nutritional value for marine fish larvae.

These three organisms, baker's yeast and *Chlorella* of marine or freshwater origin, differ markedly in fatty acid composition and the fatty acids of the rotifers to which they are fed reflect this composition. Baker's yeast has a simple fatty acid spectrum with high amounts of monoethylenic fatty acids 16:1 and 18:1 and no (n-3) series highly unsaturated fatty acid

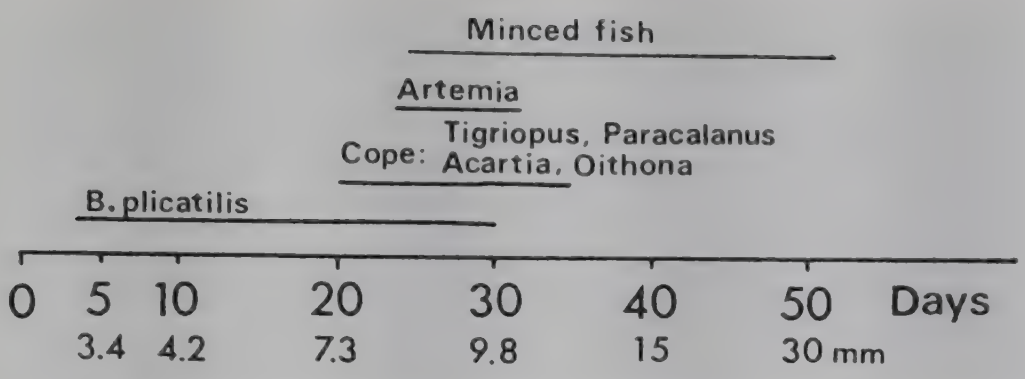


Fig. 10. Food schedule used most extensively in the production of larvae of various fish in Japan.

Marine *Chlorella*, on the other hand, has a high level of 20:5(n-3), whereas freshwater *Chlorella* has high levels of 18:2(n-6) and 18:3(n-3) — fatty acids that the rotifer is not able to chain elongate and further desaturate. Thus, except when rotifers were cultured on a marine *Chlorella*, they lacked the highly unsaturated (n-3) series fatty acids, most notably 20:5(n-3), that are essential to many marine fish. Larval marine fish, if reared on such rotifers, rapidly became deficient in essential fatty acids and mass mortalities ensue. Consequently, the fatty acid composition of rotifers chiefly determines their value as a living food for marine fish larvae.

There are a number of ways of ensuring that *Brachionus* with a suitable fatty acid composition are obtained, namely (1) culture them on a marine *Chlorella*; (2) if baker's yeast has been used, the rotifer should be secondarily cultured with marine *Chlorella* for more than 6 hours; (3) use a baker's yeast that has been grown in a culture medium containing fish oil or cuttlefish oil, such a yeast (designated yeast) has a high content of lipid and highly unsaturated, long-chain (n-3) series fatty acids; and (4) homogenize lipids, containing 20:5(n-3) and other highly unsaturated long-chain fatty acids, with a small amount of raw hen's egg yolk and water and provide the resulting emulsion directly to rotifers together with baker's yeast. Rotifers take up the lipids very easily and highly unsaturated (n-3) series fatty acids reach maximum concentration at 6–12 hours. The third and fourth methods may be used to improve the nutritional value of other living feeds. Such feeds will also take up, from the culture medium, not only highly unsaturated fatty acids but also fat-soluble vitamins.

The fatty acid composition of *Artemia nauplii* may vary in a manner similar to that of *Brachionus* depending upon the source of the *Artemia* eggs and the organism upon which they are reared. *Nauplii* reared from eggs from different locations have been shown to differ in their fatty acid content, being rich in either 18:3(n-3) or 20:5(n-3). Only the latter *nauplii* are suitable for rearing marine fish larvae. The alga *Isochrysis galbana*, because of its high content of 20:5(n-3) and 22:6(n-3), is a good organism upon which to rear *Artemia* intended as food for marine fish larvae. Watanabe et al. (1983) have shown that either of the third or fourth methods can be used to improve the nutritional quality of *Artemia* and that they should be applied to ensure an adequate essential fatty acid intake for the larvae.

Watanabe et al. (1983) measured the amino acid composition of a number of larval food organisms and did not find any marked differences

between them. All had a balanced spectrum of essential amino acids. The digestibility of the protein of rotifers was 89–94% irrespective of whether the culture organism used was baker's yeast or freshwater or marine *Chlorella*. *Artemia* protein was slightly less digestible than that of *Brachionus* for both carp (83%) and rainbow trout (89%). Values for PER and NPU of living feeds (*Brachionus*, *Artemia*, *Tigriopus*, *Moina*, and *Daphnia*) measured with carp and rainbow trout were also high and it is clear that all of these organisms are valuable protein sources. The gross energy of the live feeds was proportional to their lipid content, being high in *Brachionus* cultured with marine *Chlorella* and low in *Acartia*.

Formulated Larval Diets

Rearing techniques for various species of fish and methods for mass production of living feeds have advanced appreciably in recent years and, partly as a consequence, the number of fish species in commercial production has increased every year. Mass propagation of larval fish, however, is wholly dependent upon various species of zooplankton. The rotifer *Brachionus plicatilis* is used extensively as the initial live food for rearing larval fish. Without mass culturing of rotifers, rearing of larval marine fish would not be possible despite the fact that it is now 18 years since rotifers were first found to be suitable as live foods for larval fish.

Mass culturing of rotifers and the marine *Chlorella* upon which they feed requires not only extensive personnel and costly equipment but it is also dependent upon conditions in the natural environment. For example, the mass propagation of one million red sea bream fry requires 50×10 individual rotifers each day. Four 200-ton tanks are necessary to produce this quantity of rotifers. Large tanks are also required to culture the large amounts of marine *Chlorella* necessary as food for rotifers and the mass culture of *Chlorella* is greatly affected by weather conditions.

Thus, the rearing of larval marine fish from first feeding using artificial diets alone would be a big step toward efficient marine fish cultivation. This objective, however, to provide a consistent, reproducible technique capable of being scaled up from the laboratory to the hatchery level, remains distant. Artificial larval diets must (1) contain all the nutrients required by larval fish; (2) be digested and absorbed by the fish; (3) float in the water for 30 min without significant leaching of nutrients occurring; (4) after absorption of water, be of a size that is capable of being ingested; and (5) be acceptable to the larva (olfaction/taste).

In general, three methods have been applied to produce diets attempting to meet these criteria. (1) Binding agents with absorptive and adhesive properties, many of which are complex carbohydrates, some with ion exchange properties, and proteins have been used. (2) Microencapsulation techniques in which liquid or solid diets are encapsulated in a polymeric matrix have been employed. This may be synthetic (e.g., nylon), in which case the capsule would have to be broken to release the proteinaceous contents (the protein chains generally being cross-linked by one of a number of possible agents). The peptide bonds would thus be subject to enzymatic hydrolysis and the capsule itself nutritious. (3) Coating the particulate diet with a lipid layer that would render the particles impervious to water and at the same time be digestible and nutritious has been tried.

On occasion, these methods have been combined, in that major components in a diet may be provided in particulate form with vitamins present in separate microcapsules. Watanabe used this approach, binding diets with either alpha-starch or hydroxypropylcellulose while the vitamin mixture and lipids were microencapsulated by dextrin capsules (Celdex N). These diets were not suitable for posthatched larvae, however, although they were effective for postlarvae of body length greater than 7 mm. As shown in Table 30, dietary ingredients such as vitamins and lipids leached from the diets during 30 min suspension in the tanks, resulting in contamination of the rearing water. With postlarvae of body length greater than 7 mm, the diets were actively accepted by the larvae immediately after feeding and little nutrient leaching or contamination of the water occurred.

Subsequently, Watanabe used diets bound with calcium alginate or egg albumin microcapsules and microspheres. With both methods of diet preparation, the mixture of dietary ingredients was required to be water miscible; thus, raw hen's yolk, beef-liver homogenate, and krill homogenate, and salmon-egg extracts were typical constituents. These diets were also inadequate as a single feed for larvae of red sea bream immediately after hatching, apparently because of low digestibility. In postlarvae of *Ayu* of body length greater than 7 mm, the diets were effective although inferior to rotifers.

Kanazawa et al. (1982), at the University of Kagoshima, have prepared nylon-protein microencapsulated diets, microcoated diets in which the coating was cholesterol-lecithin, and microparticulate diets bound with carrageenan, zein, or other materials. Such diets were used in the attempted rearing of a number of species, including *Ayu*. The preparations of typical microparticulated and microcoated diets are shown in Figs. 11 and 12 and components of typical diets are listed in Table 31.

When nylon-protein microencapsulated diet alone was given to newly hatched *Ayu*, high larval mortality occurred along with an absence of growth. Subsequently, newly hatched larvae were maintained on rotifers for

Table 30. Quantitative changes in dietary nutrients during suspension in water for 30 min.

Ingredient	HNM-3	Celdex	HPC 1%	HPC 2%
Vitamin B_2(g/g)				
Initial	232.8	249.3	334.7	196.9
After 30 min	3.4	7.4	29.2	3.9
Decrease or increase (%)	−98.6	−97.0	−91.3	−98.0
Crude lipid (%)				
Initial	19.0	10.4	9.4	8.7
After 30 min	24.4	13.5	5.5	7.8
Decrease or increase (%)	+28.4	+29.8	−41.5	−10.3
Crude protein (%)				
Initial	44.6	43.7	53.1	64.1
After 30 min	25.8	72.4	46.8	72.1
Decrease or increase (%)	−42.1	+65.7	−11.9	+12.4
Total of three highly unsaturated fatty acids (%)				
Initial	0.4	2.9	3.2	2.4
After 30 min	0.9	2.9	3.1	2.1

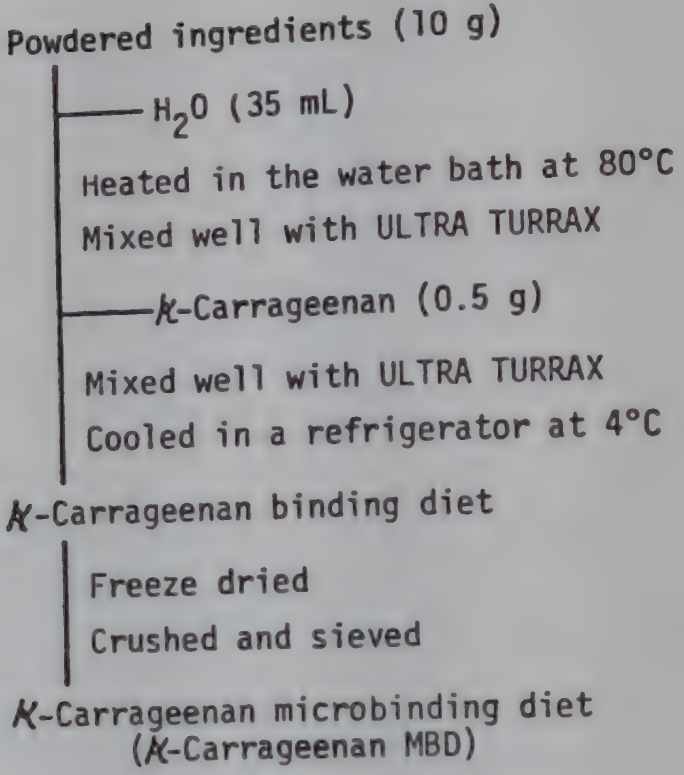


Fig. 11. Preparation of a microparticulated diet bound with carrageenan.

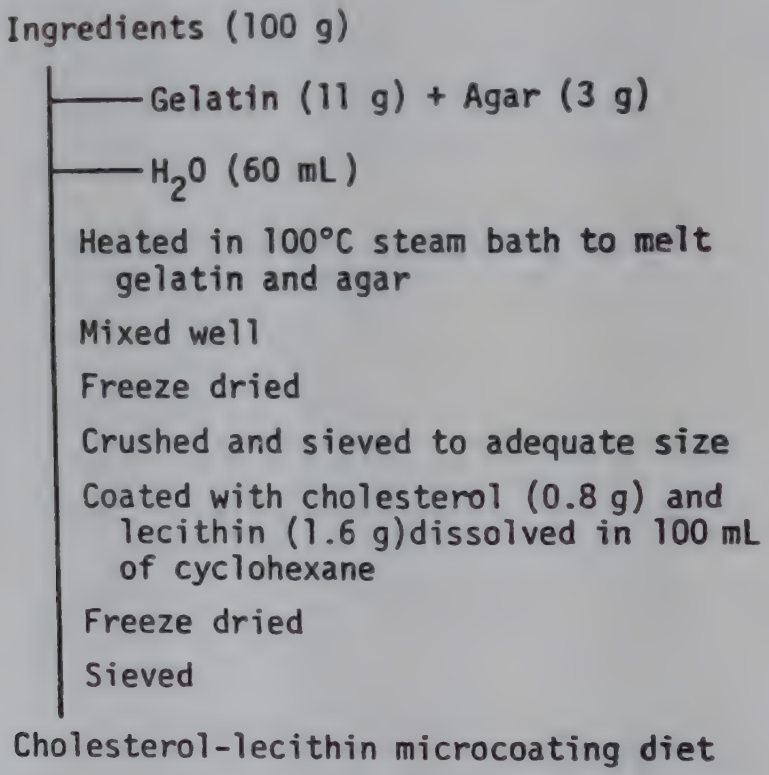


Fig. 12. Preparation of a microcoated diet, the coating being cholesterol-lecithin.

Table 31. Composition (%) of a microcoated diet (diet A) coated with cholesterol-lecithin and a microparticulated diet (diet B) bound with carrageenan.

	Diet A	Diet B
Chicken egg-yolk powder	26	29
Short-necked clam extract	17	18
Bonito extract	17	18
Casein	17	18
Gelatin	9	—
Vitamins	4	4
Minerals	4	4
Pollock-liver oil	4	4
Agar	2	—
Carrageenan	—	5

10 days after hatching. When these were then fed the nylon-protein microencapsulated diet alone for 40 days, the survival rate was 8% and when given the diet together with the rotifers over 10–50 days, the survival rate was 36%. These results do not suggest that the *Ayu* larvae utilize the nylon-protein microencapsulated diets to an appreciable extent.

The cholesterol-lecithin coated diet (Table 31, diet A) used alone from the beginning sustained growth for 50 days, although survival levels by this time were very low (3%). Growth was enhanced when larvae that had been reared for the first 10 days on rotifers were given either diet A alone or diet A together with rotifers. Survival rates for these two groups at 70 days were 13 and 25%, respectively, rates that were inferior to the control group.

Good survival and growth rates were obtained when the carrageenan-bound microparticulated diet was given to larval flatfish. The survival rate at 21 days was 27.2% (control 60.2%) and the size was 6.13 mm (control 6.54 mm).

Other artificial larval diets prepared by Kanazawa were zein-bound particles and chitosan microcapsules. The composition of these diets together with another cholesterol-lecithin microcoated diet is shown in Table 32. When used to rear *Ayu* (Fig. 13), the chitosan microcapsules scarcely supported growth and survival, but the zein-bound particles and the cholesterol-lecithin coated diet supported growth and survival to some extent over 0–60 days. These diets were especially effective in supporting growth and survival of *Ayu* larvae that had been reared for the first 10 days on rotifers, the zein diet in particular gave survival rates (65%) close to controls.

Techniques of larval diet preparation are advancing slowly and the quality of artificial feeds is likewise improving, but it may take many years before a laboratory technique of sufficient reliability has evolved to warrant its scaling up to the practical hatchery level.

Nutrition Experimentation: Approach and Design

Phase I

The development of efficient diets for aquaculture is a long-term process requiring a great deal of nutrition research and experimentation. Ideally, the nutrient requirements of the species of fish under study and the digestibility

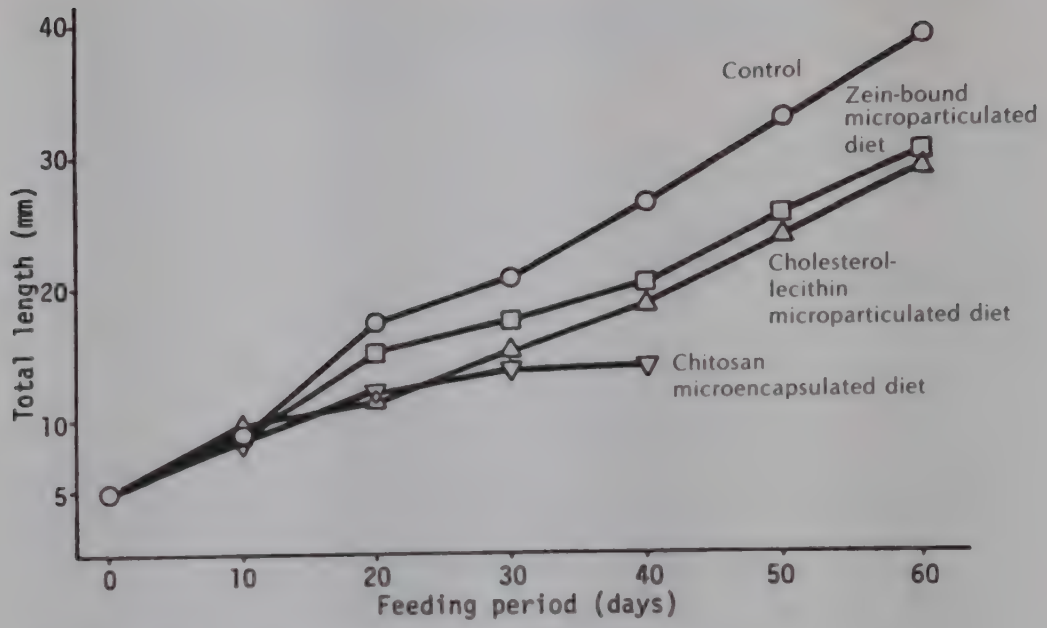


Fig. 13. Growth of larval Ayu *fed on microparticulated diets.*

of nutrients in the feedstuffs should be known before the formulation of balanced diets for fish production is attempted. However, in most cases, including domestic animals in earlier days, this information was, for many years, unavailable. Production diets, on the other hand, are usually required immediately. Furthermore, it is necessary to have some sort of practical test diets (or basal diets) that can support acceptable growth to investigate and obtain required information through applied research.

Diets, therefore, are formulated using data such as diet formulae for other fish and the composition of available natural foods and fish carcasses. This approach can produce workable diets for initial experimentation and as a

Table 32. Composition (%) of cholesterol-lecithin coated, zein-bound, and chitosan microcapsule diets.

	Cholesterol-lecithin	Zein	Chitosan
Egg-yolk powder	28	28	28
Tapes extract powder	10	10	10
Bonito extract powder	5	5	5
Casein	20	20	20
Yeast powder	10	10	10
Lecithin (soybean)	6	6	6
Vitamins	6	6	6
Minerals	5	5	5
Amino acids	5	5	5
Pollock-liver oil	5	5	5
Agar	3	—	—
Gelatin	11	—	—
Cholesterol	0.8	—	—
Lecithin	1.6	—	—
Zein	—	20	—
Chitosan	—	—	5

starting point for aquaculture production. The composition of natural foods consumed and the carcass composition of the fish give a certain amount of information about the nutritional requirements of fish. Modifications of formulae devised for fish other than that species being cultivated can also be employed to develop other suitable diets.

The next important step is evaluation of the nutritional value of local feedstuffs. The chemical composition, digestibility, and organoleptic and physical properties of selected ingredients must be determined before incorporation into the diet formulae.

Much of this information is available from animal nutrition research institutes and local animal feed mills. Once this information has been obtained, several diets can be formulated for preliminary experiments (Fig. 14). This type of diet experiment can be repeated by changing the levels of a particular ingredient or substituting one ingredient for another (Cho et al. 1974). Completion of these preliminary experiments will provide information on the growth patterns of the fish, expected live weight gain, and feed intake and efficiency. They will also provide important information on necessary experimental procedures, diet processing, equipment required, and staff training.

Some of the experimental diets that give satisfactory results in these preliminary experiments may then be tested in the field and the diet formulae test-manufactured at a local commercial feed mill. This first phase of diet development may take 2–4 years and will provide valuable information for progressing to the second phase of diet development and the commencement of nutrition research.

At the completion of phase I of the program, therefore, the proximate composition of natural foods, fish carcasses, and many potentially useful local ingredients should be known. The digestibility study of important local ingredients should be in progress and the feed processing facility (grinding, mixing, pelleting, and crumbling) should be in place (Fig. 15). The basic experimental procedures and growth pattern of the fish species under investigation will have been established and staff training should have been completed.

Phase II

Based on the results of the preliminary experiments (diet formulae, growth, and feed efficiency, etc.), experiments can be designed to confirm the results obtained from phase I and to improve still further the diet formulae. In the meantime, as various applied scientific data accumulate, the necessity for fundamental nutrition research will increase. The requirements for some essential nutrients (protein, amino acids, lipid, fatty acids, vitamins, and minerals) and the protein/energy balance must be investigated to refine the optimal dietary regimes for improved growth, better feed efficiency (Cho et al. 1976), and more economical diets for fish production. To conduct the required nutrition research, a biochemical laboratory facility and advanced staff training are an absolute necessity. Before acquiring a biochemical laboratory and training staff, however, it is important to take into consideration local factors such as human resources, chemical and laboratory supplies, equipment servicing, and the extent to

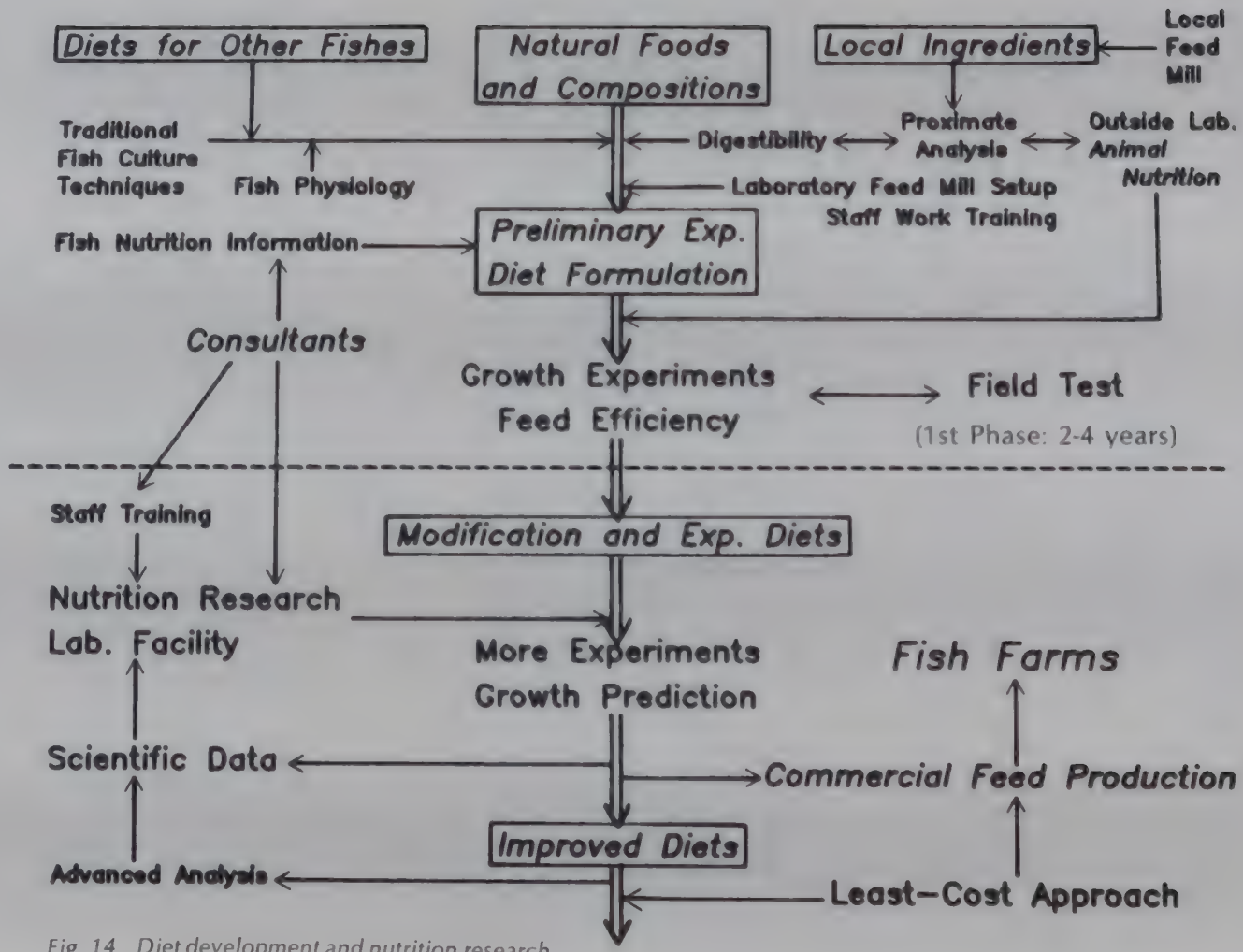


Fig. 14. Diet development and nutrition research.

which such a laboratory facility will be used. In many cases, making arrangements for analytical services with another institution or a reliable commercial laboratory is more efficient, in terms of quality, and less expensive in the initial developmental stage. The most critical concern is the optimum utilization of the resources available at any given time. Development of the laboratory facility and the necessary staff training could be phased in more slowly and the resources utilized for more experimental and extension work in nutritional and husbandry areas.

As limiting essential nutrients in the diets are identified and the balance between protein and energy is optimized, the diets should be manufactured on a commercial scale and tested at the fish-farm level. This transfer of

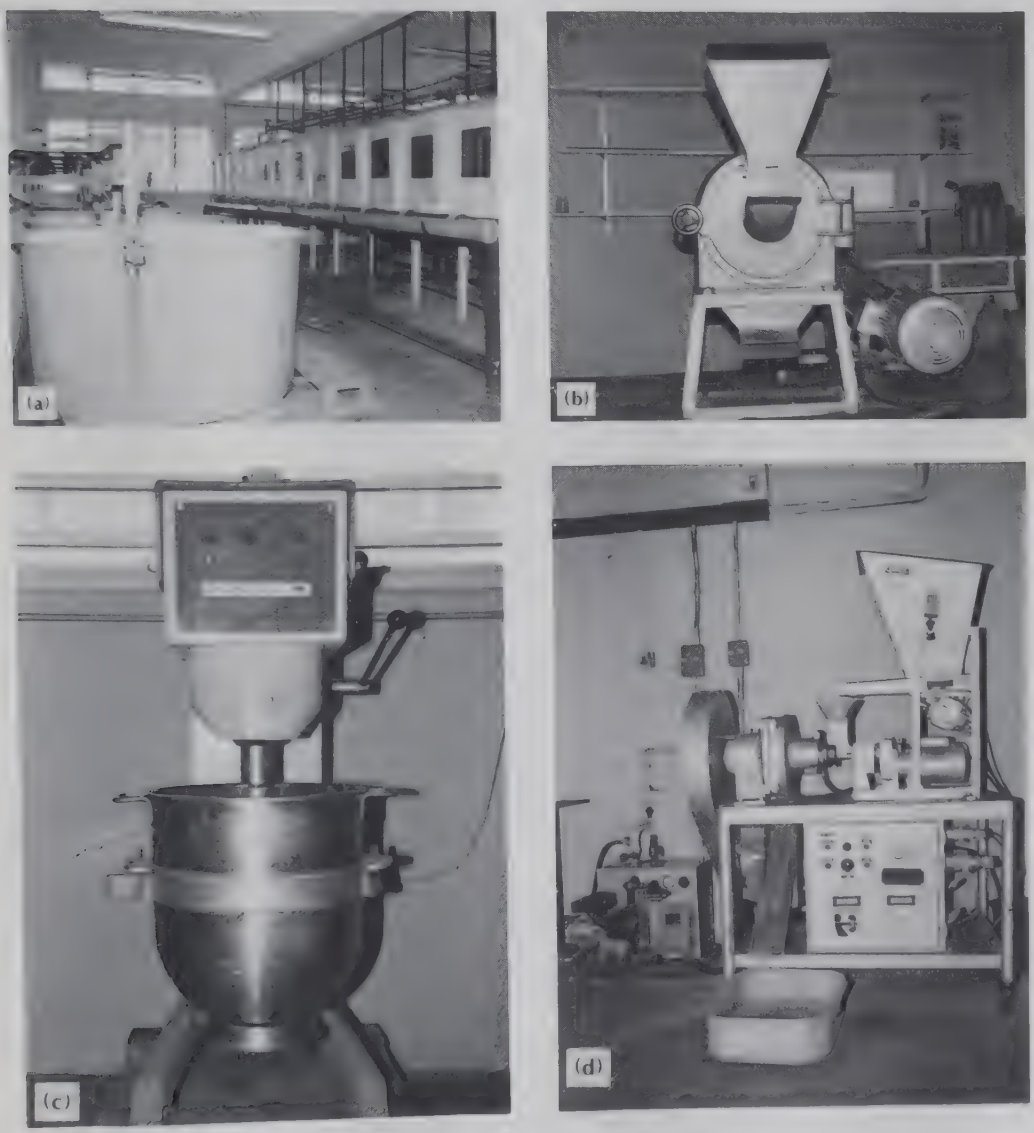


Fig. 15. Fish nutrition laboratory showing (a) fish tanks; (b) grinder; (c) mixer; and (d) pellet mill with electric steam generator. (All photos taken in the Aquaculture Unit, Primary Production Department, Singapore, and supplied courtesy of Renee Chou and Leslie Cheong.)

technology to the fish-culture industry should, in turn, lead to a continuous exchange of information between the industry and research laboratories (Fig. 14). At the same time, the feasibility of the "least-cost" approach to diet formulation can be examined for optimum utilization of locally available and imported ingredients to achieve maximum production and benefits.

Experimental Designs and Procedures

Carefully designed experiments with well-planned sample and data collection will improve the validity of the results and simplify interpretation of the data. The objectives of nutrition experiments should be specific and it is advisable to avoid attempting to answer more than one question in the same experiment. The statistical design and testing of the experimental results should be thoroughly verified prior to experimentation.

The water temperatures are frequently recorded or controlled, if necessary, because the growth performance of poikilotherms is dependent upon water temperature. Some experiments should be carried out under naturally varying water temperatures as these are the conditions under which fish will be farmed.

The duration of experiments for diet development must be long enough to increase the body weight of the control group to approximately 10 times that of the initial body weight.

The measurement of the feed intake of the fish is quite subjective and, in most cases, the amount of feed offered has to be assumed to be the amount of feed consumed. Therefore, feeding of the fish should be regarded as one of the most important activities of the nutrition experiment. The feed intake should be calculated in the following manner to compensate for the amount of feed consumed by fish that later die during the experimental period.

$$\text{Feed intake} = \frac{\text{Total feed intake}}{\text{Total fish-days}} \times \text{no. of days/period} \times \text{no. of fish}$$

The measured feed intake and composition of diets determine the amount of nutrients taken in by the fish, except for some mineral elements that may be taken up from the water, especially in the case of marine fish. Hence, all experimental diets are formulated on the basis of digestible nutrients and energy (not total nitrogen and gross energy) in relation to the control diet. Also, the ingredients and diets must be sampled immediately after processing and stored for analysis.

Growth rate is measured by weighing the fish in each tank every 2–4 weeks, so that live weight gain and feed conversion (feed/gain ratio) can be calculated. Live weight gain should be expressed on a weight/time basis rather than percentage gain, which only provides the relationship with respect to the initial body weight.

Similarly, mortality is also expressed in relation to the total fish-days per treatment rather than as a percentage of the fish population. For example, 5% mortality occurring in the first week of the experiment is quite different from 5% in the last week of the experiment, both biologically and mathematically. At the end of most dietary experiments, the initial and final carcasses should be analyzed to calculate the nutrient retention efficiencies (mainly protein, fat, and energy) to verify the live weight gain.

Growth performance of the experimental diets is compared with that of the control diet; therefore, a control diet that gives satisfactory growth and feed efficiency is essential for nutrition experiments. The conclusions of many experiments may be invalid because this comparison is made with control diets that give poor performance. The growth curve in Fig. 16 shows the different growth rates of control and test diets during a 24-week period. It can be seen that the control group of fish fed on a herring/soybean meal diet experienced greater body weight gains over the 24-week period than the experimental group, which received a mainly soybean meal diet. After 12 weeks, the experimental group was divided into two smaller groups, one of which continued to receive the soybean-based diet while the other began receiving the control diet. The growth rates of the group that began receiving the control diet increased compared with that of the control group. This type of experiment, therefore, demonstrates the need for an adequate control diet.

A checklist for an experimental setup is provided in Table 33. Before starting an experiment, it is important to ensure that the aquatic system and equipment are in good condition and that they have been cleaned and disinfected thoroughly. An inventory should then be made of all diet ingredients, vitamins, and minerals on hand and, if necessary, ingredients in short supply should be ordered. Once all ingredients have been gathered together, the experimental diets can be mixed and pelleted. Samples of the ingredients and experimental diets for analysis must be taken on the same day as mixing.

The fish for the experiment must be allowed to acclimatize in the tanks for at least 1 week before the experiment starts and the water temperature

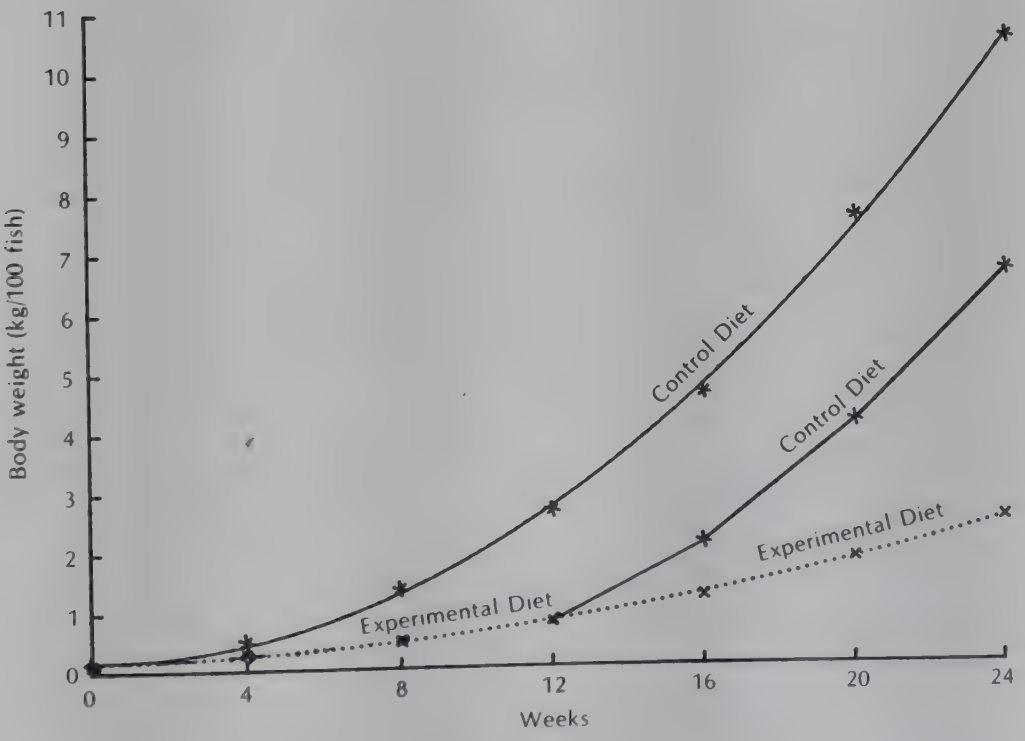


Fig. 16. A principle of comparing growth rates of control and test diets.

Table 33. Experimental setup checklist.

1. Make sure that the system is repaired, cleaned, and disinfected.
2. Ingredients inventory. Order ingredients if necessary.
3. Mix and pellet/crumble experimental diets. Diets and ingredients sampling.
4. Count fish (at least 1 week before experiment to start).
5. Set desired water temperature.
6. Randomize fish tanks. Label feed containers.
7. Initial carcass sampling. Equalize initial body weight per tank (coefficient of variance < 3%).
8. Prepare record sheets.
9. Measure feed intake and final body weight each period. Biological sampling as required.
10. Final carcass sampling and other data collection.

should be adjusted to the desired setting unless, in the case of temperate fish, varying natural water temperatures are being used. Just before the start of the experiment, the fish tanks should be randomized, with respect to the type of diet to be fed, and the body weight per tank equalized to within a coefficient of variation of 3%. Initial carcass samples should also be taken at this time. During the experiment, feed intake and live body weight should be recorded on prepared record sheets every 2–4 weeks and biological samples taken as required over the course of the experiment. At the end of the experiment, carcass samples should be taken for analysis. While these samples are being analyzed, the collected growth data should be summarized and tested for statistical significance according to the original null hypothesis of the experimental design.

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Part II

Proceedings of the Asian Finfish Nutrition Workshop held in Singapore, 23–26 August 1983

Editorial Note

The research papers presented deal with a variety of questions that are important for countries within the region. They identify problems that require resolution if finfish culture is to develop rapidly.

Some of the papers present preliminary results and further investigations will be pursued. The material, therefore, has been reproduced as submitted without formal peer review or editing.

Performance of Various Fish-Meal Diets in Young Sea Bass (*Lates calcarifer* Bloch)

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A 184-day experiment was conducted on young sea bass, *Lates calcarifer* Bloch (mean weight 19.1 g), raised in a seawater flow-through system. The fish were fed three isonitrogenous and isocaloric diets (crude protein, 54.7 ± 0.9%; ether extractable fat, 8.4 ± 1.0%; carbohydrate, 12.5 ± 0.4%) formulated from Norwegian herring meal and fish meals processed in Thailand and Singapore. Deboned fish was used as the control diet. The Singapore fish-meal diet was more acceptable than the other diets and gave significantly better growth by weight. Protein efficiency ratios obtained with the Singapore and Norwegian fish-meal diets were significantly higher than those obtained with the Thai fish-meal and control diets. Increasing the protein level in the Singapore and Norwegian fish-meal diets did not significantly improve growth response. In general, Singapore fish-meal diets gave the best weight increments. Mean weights of the fish increased by 132.6 – 204.7 g after 184 days, depending upon the diet. Singapore fish meal is recommended for use in formulated feeds that can serve as alternatives to the trash fish presently used by Singapore fish farmers.

Introduction

Fish meals available in Singapore are either locally produced or imported. Because they vary in price, composition, and quality, this can pose problems in their formulation. Fish meals with low protein content (< 52%) are normally of low nutritive value and low digestible protein. This may be a result of using large proportions of small fish, fish heads and tails, overheating during processing, or improper storage conditions (Tan and Lee 1975).

The aim of this experiment was to compare the performance of three isonitrogenous and isocaloric fish-meal diets in young sea bass, *Lates calcarifer* Bloch (mean initial weight 19.1 g). The fish meals used were processed in Norway, Thailand, and Singapore. Two additional diets, with the highest possible protein levels, were formulated with Singapore and Norwegian fish meals and tested to ascertain whether or not growth response could be improved further.

Materials and Methods

Sea bass fingerlings (total length 10 cm), caught from the wild in Thailand, were used in the experiment. The fish were weaned over a 2-week period from trash fish to fish-meal pellets. The weaning diet contained equal portions of Singapore, Thai, and Norwegian fish-meal pellets. The weaned sea bass (mean weight 19.1 g) were stocked at random in 18 fibreglass tanks (80 cm × 50 cm × 60 cm; 240-L capacity; seawater volume of 160 L) with 15 fish/tank. The tanks, which were arranged in series, were continuously supplied with filtered seawater at a rate of 2.5 L/min·tank^{-1} (23 changes/24 hours). Seawater (salinity 30 ± 2‰) was pumped from two 2.5 m^3 header tanks through two filters (diameter 26 cm; with sand medium) by two self-priming centrifugal seawater pumps (1.5 hp) (only one pump was used at any one time) and delivered to the tank system. The water temperature was 28 ± 2°C.

The fish, which were held in the system for 184 days, were measured every month. Lengths (total and standard) and weights were recorded to the nearest 0.1 cm and 0.1 g respectively. Quinaldine was used to anaesthetize the fish. Excess water was removed with a soft damp cloth.

The study was conducted with two feed groups. In the first group, three diets (B, C, D) having similar protein, fat, and carbohydrate levels (54.7 ± 0.6%; 8.4 ± 1.0%; and 12.5 ± 0.4% respectively) were formulated from Norwegian, Thai, and Singapore fish meals (Table 1). Table 2 presents the calculated amino acid levels in the diets. Deboned threadfin snapper (*Nemipterus* spp.) was used as the control diet (A).

In the second feed group, two diets (B^1, D^1) were formulated from Norwegian and Singapore fish meals to achieve the highest possible protein levels (66 and 57% respectively). The diets were tested against diets A, B, C, and D.

Table 1. Chemical composition of experimental diets.

	Diet				Mean
	A	B	C	D	(B,C,D)
Protein source Country of origin	Fish meat	Fish meal Norway	Fish meal Thailand	Fish meal Singapore	
Chemical composition (percentage dry basis)					
Crude protein (N × 6.25)	91.1	53.8	55.5	54.9	54.7±0.9
Ether extract	4.7	8.4	7.4	9.4	8.4±1.0
Crude fibre	0.1	5.8	0.7	2.9	—
Ash	5.4	12.4	12.5	20.6	—
Calcium	(0.2)	(2.9)	(2.9)	(5.1)	—
Phosphorus	(0.4)	(2.0)	(2.0)	(3.0)	—
Carbohydrate (calculated)	Nil	12.7	12.7	12.1	12.5±0.4
Calculated energy					
Digestible energy[a] (kcal/kg dry diet)	4067	3416	3394	3526	3445±71

[a] Calculated as in Teng et al. (1978) for grouper, using rainbow trout values of 4 kcal/g protein, 9 kcal/g lipid, and 4 kcal/g carbohydrate.

Table 2. Calculated amino acid levels in experimental diets.

Components (g/kg dry diet)	Diet			Dietary requirement (g/kg dry diet)[a]		
	B	C	D	Chinook salmon	Gitlhead bream	Japanese eel
Crude protein	538	555	549	—	—	—
Nonprotein nitrogen	32	160	75	—	—	—
Total amino acids	506	395	474	—	—	—
Essential amino acids[b]	228	216	221	137	—	—
Arginine	36	34	34	24	<10.4	17
Histidine	16	20	9	7	—	8
Leucine	38	33	36	16	—	20
Isoleucine	18	17	18	9	—	15
Lysine	47	48	48	20	20	20
Methionine	8	6[c]	7	16[c]	16	12
Phenylalanine	21	25	24	21[d]	—	22[d]
Threonine	24	14	22	9	—	15
Tryptophan	—	—	—	2	2.4	4
Valine	20	19	23	13	—	15
Nonessential amino acids						
Tyrosine	16	8	12	—	—	—
Glycine	31	34	32	—	—	—
Alanine	35	31	32	—	—	—
Aspartic acid	52	34	47	—	—	—
Glutamic acid	80	58	78	—	—	—
Cystine	1	—	2	—	—	—
Proline	39	—	29	—	—	—
Serine	24	14	21	—	—	—

[a] Source: Cowey (1978).
[b] Source: Yone (1976) for red sea bream and Halver et al. (1957) for salmonids.
[c] In the absence of cystine.
[d] In the absence of tyrosine.

The feeds were extruded using an electric food grinder. They were dried on racks in a dehumidified room to dietary moisture levels of around 20%. The feeds were packed in plastic bags and stored in a freezer. Daily feeds were dispensed by hand from airtight bottles that were replenished every week. Pellet diameters ranged from 2.4 to 4.0 mm according to the stage of growth. The sea bass were fed to satiation three times daily (0900–1030 hours, 1130–1300 hours, and 1400–1500 hours). Feed bottles were kept in a freezer between feedings.

The chemical composition of the fish meals and feeds were analyzed by the Animal Nutrition Laboratory, Primary Production Department. Crude protein analysis was carried out using the Kjeldahl method; crude fat by ether extraction with the Soxhlet extraction apparatus; and ash with a muffle furnace. Calcium was measured using an atomic absorption spectrophotometer and phosphorus colourimetrically using a UV-visible spectrophotometer. Amino acids were analyzed by acid hydrolysis and liquid chromatography.

Results

Performance of Diets A, B, C, and D

Feeding and Utilization

Feeding and utilization data from the 184-day experiment are presented

Table 3. Performance of different fish-meal diets.

	Fish flesh	Fish-meal diet		
		Singapore	Norway	Thailand
As-fed intake (kg)	11.3±1.3	4.2±0.2	3.5±0.3	3.6±0.3
Dry intake (kg)	2.3±0.3	3.3±0.2	2.5±0.2	2.8±0.3
Total weight gain (kg)	1.8±0.3	2.8±0.2	1.9±0.3	1.8±0.2
Feed : gain	1.2±0.1	1.2±0.0	1.3±0.1	1.5±0.2
Protein efficiency ratio	0.9±0.1	1.6±0.1	1.4±0.1	1.2±0.1

Note: The levels of essential amino acids in all fish-meal diets exceed the requirements of salmonids.

in Table 3. There were significant differences among the four treatments with respect to dry feed intake, protein efficiency ratio, mean energy intake, and feed : gain ratio. An a posteriori test (Student–Newman–Keuls multiple-range test) shows that the total dry feed intake of the fish fed diet D (Singapore fish meal) was significantly higher ($P < 0.01$; 3314 g/15 fish·184 days^{-1}) than that of fish fed the other diets. Protein utilization (measured by the protein efficiency ratio) was best in sea bass fed diet D (1.6), but this value was not significantly higher than that obtained with diet B (Norwegian fish meal, 1.4). Both results, however, were significantly higher ($P<0.01$) than those obtained with diets C and A (Thai fish meal and control diet, 1.2 and 0.9 respectively).

Fish-Meal Composition

Table 4 presents the chemical compositions of the Norwegian, Thai, and Singapore fish meals used in the experiment. The latter two have lower crude protein levels and higher ash contents. The essential amino acid levels (expressed as percentage in crude protein) are comparable among the fish meals. The amino acid levels in isonitrogenous diets B, C, and D are also similar (Table 2) and are comparable with the requirements of Chinook salmon, gilthead bream, and Japanese eel (all carnivorous fish) as reported by Cowey (1978). The amount of total amino acids per kilogram dry diet was lowest in diet C, but this is mainly a result of the nonessential amino acids. The nonprotein nitrogen level is highest (160 g/kg) in diet C.

Growth

There were significant differences among the treatments with respect to normal and relative growth rates and mean increase in standard length. The multiple-range test shows that of these the normal and relative growth rates (by weight) were significantly higher in sea bass fed diet D (1 g/fish·day^{-1}; 1.3% per day). Growth, in terms of length increase, was not significantly higher in sea bass fed diet D (1 g/fish·day^{-1}; 1.3% per day). Growth, in terms of length increase, was not significantly different in the fish-meal treatments but was still numerically highest (10.9 cm) in those fish fed diet D. This value was significantly greater than that of the fish fed the control diet.

Survival and Condition

There was no significant difference in survival rates among the four treatments (58–96%). The initial condition factors were not significantly different among the treatments (24.7–26.1); final condition factors, however, differed significantly. The multiple-range test showed that the

Table 4. Chemical composition (%) of fish meals used in experiment. (Figures in parentheses are percentage in crude protein.)

	Norway	Thailand	Singapore
Moisture	7.1	9.8	5.7
Percentage dry basis crude protein (N × 6.25)	75.7	63.2	69.3
Crude fat	7.0	8.2	6.9
Crude fibre	0.5	0.6	0.6
Ash	15.4	27.4	24.8
Calcium	3.5	6.4	4.6
Phosphorus	2.8	3.5	4.3
Total amino acids	70.9	45.0	59.5
Percentage amino acids in crude protein	93.7	71.2	85.9
Essential amino acids[a]	31.9	24.6	27.7
Percentage essential amino acids in crude protein	42.1	38.9	40.0
Percentage essential amino acids in total	45.0	54.7	46.6
Arginine	5.1(6.7)	3.9(6.2)	4.3(6.2)
Histidine	2.2(2.9)	2.2(3.5)	1.1(1.6)
Leucine	5.4(7.1)	3.8(6.0)	4.6(6.6)
Isoleucine	2.5(3.3)	1.9(3.0)	2.2(3.2)
Lysine	6.6(8.7)	5.5(8.7)	6.0(8.7)
Methionine	1.1(1.5)	0.7(1.1)	0.9(1.3)
Phenylalanine	2.9(3.8)	2.8(4.4)	3.0(4.3)
Threonine	3.3(4.4)	1.6(2.5)	2.7(3.9)
Valine	2.8(3.7)	2.2(3.5)	2.9(4.2)
Nonessential amino acids			
Tyrosine	2.2(2.9)	0.9(1.4)	1.5(2.2)
Glycine	4.4(5.8)	3.9(6.2)	4.0(5.8)
Alanine	4.9(6.5)	3.5(5.5)	4.1(5.9)
Aspartic acid	7.3(9.6)	3.9(6.2)	5.9(8.5)
Glutamic acid	11.2(14.8)	6.6(10.4)	9.8(14.1)
Cystine	0.1(10.1)	Negligible	0.2(0.3)
Proline	5.5(7.3)	Negligible	3.6(5.2)
Serine	3.4(4.5)	1.6(2.5)	2.7(3.9)

[a] As determined for salmonids by Halver et al. (1957) and red sea bream by Yone (1976).

final condition factors in fish fed diets B and D were significantly higher than those in fish fed the control diet.

Performance of Diets B[1] and D[1] Compared with Diets A, B, C, and D

Feeding and Utilization

There were significant differences among all six treatments with respect to dry feed intake, feed : gain ratios, and protein efficiency ratios. The multiple-range test showed that the dry feed intakes of sea bass fed diets D[1] and D were significantly higher (3081 and 3414 g/15 fish·184 days^{-1}) than those of sea bass offered Norwegian fish-meal diets B[1] and B (2184 and 2497 g/15 fish·184 days^{-1}). The difference between dry feed intakes of fish fed the same type of fish meal, however, was not significant (i.e., BB[1] and DD[1]).

Feed : gain ratios for fish fed diets B, D, and D[1] (1.3, 1.2, and 1.3) were not significantly different among themselves but were all significantly higher than the feed : gain ratios obtained with diet B[1] (1.0). The protein efficiency ratio with diet B[1] (1.6), however, was not significantly higher than

that obtained with diet B (1.4), even though diet B¹ contained 11% more crude protein. A similar observation was made with respect to the protein efficiency ratios of fish fed diets D and D¹ (1.2 and 1.5). In this case, the protein level of diet D¹ was only 2% higher than that of diet D.

Growth

Growth, in terms of length and weight, was not significantly higher in those fish fed diets B and B¹ or D and D¹.

Survival and Condition

The diets did not significantly affect sea bass survival rates (57.8–95.5%). The final condition factors of sea bass fed Norwegian and Singapore fish-meal diets did not vary significantly with the same fish meal (i.e., BB¹ and DD¹). The final mean condition factor was slightly poorer (24.8) than the initial mean condition factor (25.3); otherwise, the sea bass were healthy throughout the experiment and devoid of skin lesions and other external signs of sickness.

Discussion

In general, the performance of Norwegian and Singapore fish-meal diets was better than that of the Thai fish-meal and control diets. The dry feed intake of sea bass fed the Singapore fish-meal diet (D) was significantly higher than that of sea bass fed the other diets. Because diets B, C, and D were nearly isonitrogenous and isocaloric, it can be concluded that diet D, which was consumed in significantly larger quantities, was the most attractive to the fish. Observations made during feeding confirmed that the sea bass responded best to this diet. One reason for this marked preference may be that the Singapore fish meal, processed locally from trash fish caught nearby, was of better quality than the other two fish meals.

Protein efficiency ratios were not significantly different between diets B and D. Growth by weight was, however, significantly higher in fish fed diet D. Figure 1 shows that the mean weight of the fish fed this diet was consistently higher over fixed periods. This indicates that the sea bass preferred, and ate more of, diet D and converted it into a proportionate weight gain. They did not, however, increase significantly in length, although the increase was highest for those fish fed diet D.

In comparison, diet C gave a significantly lower protein efficiency ratio than diets B and D, but feeding and growth responses of the fish were comparable to those fed diet B. The essential amino acid levels in all three diets are comparable (Table 2), but the biological availability of the essential amino acids is unknown and chemical analysis of the feed does not really indicate the quality of the fish meal used (C.Y. Cho, personal communication). The nonprotein nitrogen fraction of diet C (160 g/kg dry diet) was the highest among the fish-meal diets, whereas the total amino acids were the lowest (395 g/kg dry diet). These factors could also have accounted for the relatively poor performance of diet C.

Deboned fish was not a good diet to use because of certain nutritional deficiencies (e.g., minerals; Table 1). The fish meat was also less water stable than the fish-meal pellets and easily polluted the water.

Increased amounts of the Norwegian and Singapore fish meals in the diet maximized crude protein levels but did not elicit a significantly better

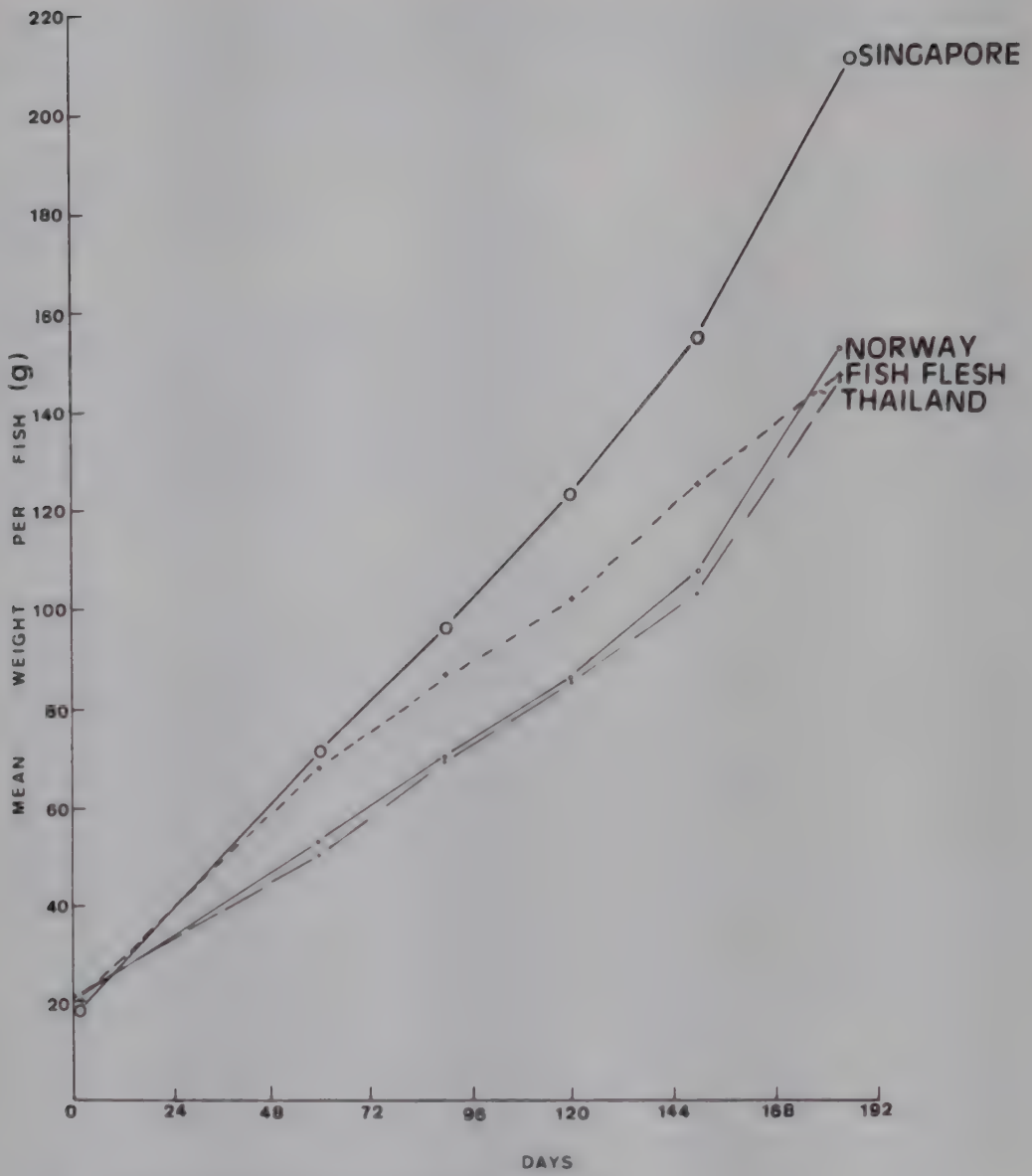


Fig. 1. Growth of sea bass fed various fish-meal diets.

growth response in sea bass. The performance of diet B^1 was, in many respects, similar to that of diet B, although their crude protein contents were 66 and 54.7% respectively. This indicates that perhaps the amount of protein in diet B^1 exceeded the requirements of the fish and the excess was burnt off as energy. The performance of diets D^1 and D was similar. This was not surprising as the crude protein level of diet D^1 was only 2% higher.

Conclusions

The results indicate that the batch of locally processed fish meal used in the sea bass experiment performed better than those fish meals from Thailand and Norway. The sea bass distinctly preferred diets compounded from Singapore fish meal and grew best on them. From a practical point of

view, i.e., in terms of nutritional adequacy, feed availability, and economic feasibility, Singapore fish meal is recommended for use in formulated feeds. These feeds have the potential to replace, either partially or completely, the trash-fish diet presently used by fish farmers.

Acknowledgments

I wish to thank Mr Leslie Cheong, Head of the Aquaculture Unit, for his suggestions and interest in this work; Miss Lee Yeng, Mr J.S. Wong, and Miss F.J. Wong for their assistance in conducting the experiment; Dr C.Y. Cho, University of Guelph/Ontario Ministry of Natural Resources, Canada, for his advice; the Primary Production Department, Ministry of National Development; and the International Development Research Centre, Canada, for their support. This study was conducted as part of the research under the IDRC/Singapore Fish Culture Project Phase II (1981–1983).

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An Evaluation of the Apparent Digestibility of Some Locally Available Plants and a Pelleted Feed in Three Finfish in Malaysia

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This paper presents some of the findings of the studies on finfish nutrition that are being conducted at the Universiti Pertanian Malaysia. Three studies have been completed in this program: digestibility of carpet grass (*Axonopus compressus*) and napier grass (*Pennisetum purpureum*) by grass carp (*Ctenopharyngodon idella*), digestibility of yam leaves (*Colocasium antiquorum*) and the 37% protein Malaysian Agricultural Research and Development Institute (MARDI) feed by Kalui (*Osphromenus gouramy*), and digestibility and digestion coefficients of the 37% protein MARDI feed by Jelawat (*Leptobarbus hoevenii*). The apparent digestibilities of carpet grass and napier grass in grass carp and yam leaves in Kalui were 20.92, 16.45, and 55.29% dry matter respectively. The apparent digestibilities of the 37% protein MARDI feed in Kalui and Jelawat were 82.85 and 70.15% respectively. The low digestibility of the plants in grass carp and Kalui indicated that, for more intensive culture systems, nutritionally balanced pellet feeds are necessary for successful culture of these fish.

Introduction

The growing demand for fish protein in Malaysia has motivated active development of aquaculture (Pathansali and Zainol 1976). Successful aquaculture projects depend on several inputs and it is envisaged that many problems facing the aquaculture industry in Malaysia, as well as in Southeast Asia (IDRC 1973), will need urgent attention. One of the constraints to the development of aquaculture is the formulation of nutritionally balanced diets to meet the requirements of the fish. In Malaysia, there are a variety of agro-based industrial wastes, such as oil palm waste, pineapple waste, and coconut waste. Efforts have been made to make use of these organic wastes in formulating feeds for animals, including fish. An important prerequisite in feed formulation, however, is information on the digestibility of feed ingredients. With this in mind, a program was established in 1978 to evaluate the digestibility of some locally available plants and pelleted feeds by finfish at the Faculty of Fisheries and Marine Science, Universiti Pertanian Malaysia. This paper will present the findings and progress of experiments conducted during the period 1978–1983.

Methods and Materials

Three separate experiments were conducted to determine: (1) digestibility of carpet grass (*Axonopus compressus*) and napier grass (*Pennisetum purpureum*) by grass carp (*Ctenopharyngodon idella*), (2) digestibility of yam leaves (*Colocasium antiquorum*) and MARDI feed (37% protein) by Kalui (*Osphromenus gouramy*), and (3) digestibility of MARDI feed (37% protein) by Jelawat (*Leptobarbus hoevenii*) and digestion coefficients of the ingredients in the feed. The mean weights of grass carp, Kalui, and Jelawat used in the studies were 1.1 kg, 50 g, and 41 g respectively.

Experimental Tank

A V-shaped tank (1.3 m diameter × 0.7 m depth) was used for the study of Jelawat and Kalui (Fig. 1). The stocking density ranged from 6 to 8 fish per tank. Running water (30 L/hour) and aeration were supplied to the tank continuously.

The grass carp were maintained in a large glass aquarium (0.75 m × 0.90 m × 2.1 m) or a large fibreglass circular tank (1.5 m diameter × 0.7 m depth). The stocking density ranged from 2 to 4 fish per tank. Running water and aeration were provided for the tank.

Test Diets and Internal Markers

Napier grass, carpet grass, and yam leaves were provided by the farm of Universiti Pertanian Malaysia, whereas the pellet feed was supplied by the

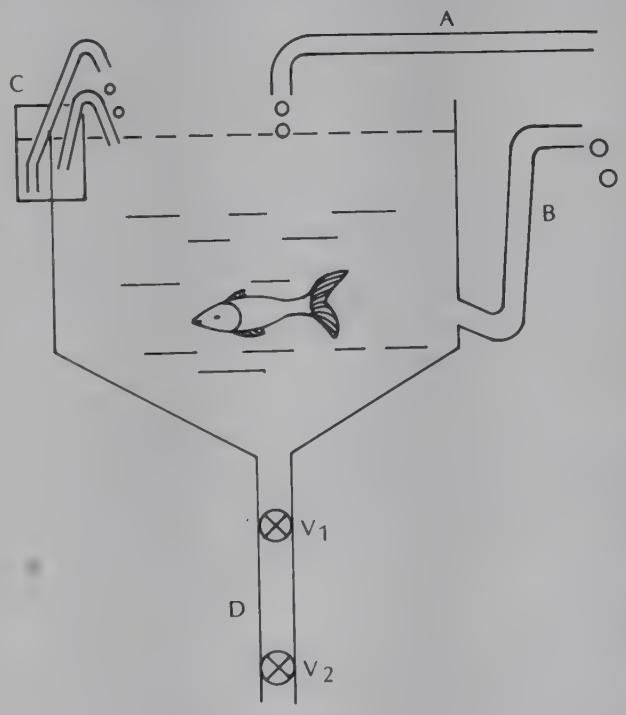


Fig. 1. Experimental tank. A, inflow of seasoned water; B, outflow of water from the tank; C, filter and aeration; D, feces collection tube; V_1 and V_2, control valves. Operation of the feces collection tube: open V_1 for 5 min and close it again; open V_2 and drain the feces into a beaker; repeat the procedure two to three times.

Freshwater Fish Research Station, MARDI, Malacca. The composition of the feed is given in Table 1. The test diet was made up of 30% of the test ingredient and 70% of the reference diet (37% MARDI feed).

The lignin content in the plant was used as the internal marker for evaluating digestibility in the fish. For the pellet feed, 1% Cr_2O_3 was incorporated as the internal marker.

Feeding of Kalui and Jelawat and Collection of Fish Feces from the Experimental Tanks

Kalui or Jelawat were fed twice daily, 0830 and 1515 hours, with pellet feed weighing about 5% of the total fish body weight. In studying the digestibility of yam leaves by Kalui, the fish were fed twice daily with an excess of yam leaves. After each feeding, the excess feed was removed and the 10 L (approximately) of water within the tank was drained to flush out any uneaten feed that had sunk to the bottom of the tank. The feces were collected from the trapping tube of the experimental tank twice daily, 0800 and 1415 hours. The collected feces were centrifuged and dried at 50°C in an oven. After drying, they were ground and subjected to a proximate analysis.

Feeding of Grass Carp and Collection of Intestinal Solids

The fish were fed twice daily with an excess of napier grass or carpet grass. Owing to the active and fast swimming character of the grass carp, the excreted feces were quickly dispersed in the water. As such, the feces of large grass carp could not be collected using the trapping tube in the experimental tank. Consequently, they were maintained in the large glass or fibreglass tanks and the intestinal solid was removed just before it was excreted as feces (Law 1978; Law and Syed Razlan 1981).

Proximate Analyses

The analyses of lignin, cellulose, lipid, and ash in the feeds and feces were carried out according to the techniques of Goering and Van Soest (1970); crude protein and dry matter followed the methods of the AOAC (1975); gross energy content was determined with a Parr adiabatic oxygen bomb calorimeter; and chromic oxide content was estimated using the method of Kimura and Miller (1957).

Table 1. Composition of the pelletized feed.

Ingredient	Percentage composition
Fish meal	55.75
Soybean	27.00
Copra cake	7.00
Maize	3.00
Rice bran	1.00
Tapioca	2.00
Vitamin mix[a]	0.60
Mineral mix[a]	3.65
Analyzed crude protein	37.6

[a] As recommended in National Research Council (1977).

Equations Used to Evaluate Digestibility

The apparent digestibilities of the dry matter and nutrients in the feed were estimated by using the following equations (Cho et al. 1982):

Apparent digestibility coefficient of nutrient or dry matter

$$= 1 - \left(\frac{\%\ \text{internal marker in diet}}{\%\ \text{nutrient in diet}} \times \frac{\%\ \text{nutrient in feces}}{\%\ \text{internal marker in feces}} \right) \times 100$$

Apparent digestibility coefficient of test ingredient

$$= \frac{100}{30} \left(\begin{matrix} \text{digestibility coefficient} \\ \text{of test diet} \end{matrix} - \frac{70}{100} \begin{matrix} \text{digestibility coefficient} \\ \text{of reference diet} \end{matrix} \right)$$

Results and Discussion

Evaluation of the Digestibility of Napier Grass and Carpet Grass by Grass Carp

The apparent digestibilities of dry matter, protein, fat, and ash of carpet grass and napier grass by grass carp are presented in Table 2. The results indicate that both grasses were poorly digested by grass carp and the apparent digestibilities of dry matter for carpet grass and napier grass were only 20.92 and 16.45% respectively. The low digestibility of grass by grass carp has rendered it necessary for the fish to consume a large amount of grass daily to meet its nutritional requirements. The poor digestibility of grasses is probably due to the poor grinding system of the fish. Microscopic examination of the intestinal content of the grass carp fed with grass revealed that the majority of the grass cell walls were not broken and remained intact, whereas the ruptured cells were all empty. This indicated that the poor grinding system of the fish might be responsible for the low digestibility of the grasses.

The study of the changes in chemical composition of the intestinal content along the fish intestine revealed that 86.7% of the released protein from the grass in the intestine was absorbed. These results indicate that grass carp have an excellent ability to absorb protein from the intestine. One should be able to increase the growth rate of grass carp by feeding them a nutritionally balanced diet instead of vegetable leaves and grasses. This aspect is being studied in our laboratory and the results seem to support this phenomenon.

Digestibility of Yam Leaf Pellet Feed Fed to Kalui (*Osphromenus gouramy*)

The slow growth rate of *Osphromenus gouramy* fed vegetables or leaves (Ong 1969) is probably due to the poor digestibility of these feeds in the fish. The nutritional requirement of this fish is not known. There is an urgent need, therefore, to understand the digestibility of locally available vegetables and leaves used in feeding this fish.

Table 2. Apparent digestibility of carpet and napier grasses by grass carp.

	Dry matter (%)	Protein (%)	Fat (%)	Ash (%)
Carpet	20.92	62.98	—	16.52
Napier	16.45	63.96	51.74	22.32

Yam leaves (*Colocasium antiquorum*) and water Kangkong (*Ipomoea aquatica*) are generally used in feeding Kalui in Malaysia. The results of the evaluation of the digestibility of yam leaves indicated that the leaves were poorly digested, the apparent digestibility of dry matter, protein, and fat being 55.29, 72.04, and 30.53% respectively. However, the fish could digest the pellet feed (MARDI, 37% protein) very well, the apparent digestibility of dry matter, protein, fat, ash, and gross energy in this case being 82.85, 90.24, 94.84, 64.02, and 84.27% respectively.

The results indicated that yam leaves could not meet the nutritional requirements of Kalui and a nutritionally balanced pellet feed should be employed in the culturing of this fish. A preliminary study of Kalui fed yam leaves and pellet feeds in cage culture indicated that the growth rate of fish fed with pellet feed (40% protein) was 7.8 times faster than those fed yam leaves (Tan 1983). These data seem to support the observation that a nutritionally balanced feed, instead of vegetable leaves, is required for mass production of Kalui.

Evaluation of the Digestibility of Pellet Feed Fed to Jelawat (*Leptobarbus hoevenii*)

Leptobarbus hoevenii is a popular food fish in Malaysia. The great demand for this fish has led to its mass culturing. The nutritional requirements of this fish are poorly understood. Therefore, evaluation of the digestibility of the MARDI feed (37% protein) by *Leptobarbus hoevenii* was undertaken at Universiti Pertanian Malaysia.

The apparent digestibilities of dry matter, protein, fat, ash, carbohydrate, and gross energy were 70.15, 85.97, 95.18, 47.19, 58.59, and 76.21% respectively.

An evaluation of the digestible value of all of the ingredients used in formulating the MARDI feed was made. The results indicated that 100% of the protein, fat, and gross energy in the fish meal was digested and copra cake meal was utilized better than soybean meal and maize (Law 1983). Copra cake is a by-product of the agro-based industry in Southeast Asia. The supply is abundant and the price is cheap. The possibility of using copra cake to replace soybean and maize for the production of a cheaper feed for the mass culture of ikan Jelawat is under investigation.

Acknowledgments

The authors would like to thank the head of the Freshwater Fisheries Research Station, MARDI, Melacca, Encik Ahmad Tajuddin bin Zainuddin and his research officer Encik S. Pathmasothy for their cooperation in providing the experimental fish and pellet feed.

The authors also wish to thank Captain Mohd. Ibrahim bin Haji Mohamad, Dean of the Faculty of Fisheries and Marine Science, Universiti Pertanian Malaysia, for his support and encouragement; the farm of Universiti Pertanian Malaysia for the supply of grasses; and Encik Poh Yong Thong, Encik Azahar bin Othman, and Zainal Abidin bin Bahari for their technical help.

The projects were funded by IDRC, International Foundation of Sweden (IFS), and Universiti Pertanian Malaysia.

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Evaluation of the Use of Internal and External Markers in Digestibility Studies

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Results of experiments in which digestibility estimates were made using indigenous components, in particular the crude fibre, hydrolysis resistant ash, and hydrolysis resistant organic matter contents, of the feed(s) as markers are summarized. The merits of using indigenous components as markers, as opposed to an artificial marker such as Cr_2O_3, are evaluated.

Introduction

Digestibility studies of feeds and the specific nutrients therein are of primary importance in animal nutrition. Such studies on fish pose greater technical problems by virtue of the fact that fish live in an aquatic medium, which brings about leaching of fecal material nutrients. Investigations of digestibility of feeds in fish are relatively recent. Most of these investigations were based on the introduction of an inert digestion marker, such as chromic oxide, into the feed — a method originally introduced for livestock by Edin (1918) that later proved to be useful for fish as well (Austreng 1981; Windell et al. 1978; Cho and Slinger 1979; Smith et al. 1980; Plakas and Katayama 1981).

Digestibility estimates based on the introduction of an inert marker into the feed become reliable only if the marker (1) does not, presumably, affect the physiology of digestion, (2) moves through the alimentary canal at the same rate as the other feed ingredients, and (3) is not absorbed. The differential rate of movement of the marker through the gut passage was recognized early and to compensate, at least partially, for errors arising due to these differences fecal material collected over a few days was pooled for analysis. Recently, however, the likely reasons for the differential passage of the marker in the digestive tract from that of the feed have been highlighted (Bowen 1978). Hilton et al. (1981) implied that extruded-pellet feed moved slower than steam-pelleted feed due to the higher density of the former. De Silva and Owoyemi (1983) showed that the specific gravity (density) of the diet affected its passage through the intestinal tract.

The following indigenous components of diets have been used in both ruminants and fish as markers in digestibility studies: silica (Hickling 1966), cellulose (Buddington 1980), hydrolysis resistant ash (HRA) (Bowen 1981; De Silva and Perera 1983), hydrolysis resistant organic matter (HROM) (Buddington 1980), and also ash, under special circumstances (De Silva et al. 1984).

In this paper, experiments carried out on the relative merits of using different indigenous markers to make digestibility estimates in the Asian cichlid *Etroplus suratensis* fed on a macrophyte, and a natural population of *Sarotherodon mossambicus* are reviewed. In addition, observations on the digestibility of artificial diets in *S. niloticus* fry, based on Cr_2O_3 as a marker, are also presented.

Materials and Methods

Although the methodology will not be presented here, in all the experiments the precautions suggested by Cho et al.(1982) in the collection of feces were taken into account. It should be noted, however, that deviations had to be adopted in the "salinity" experiments in that the fecal material had to be washed with a small amount of water to remove the adhering salts.

Chemical analyses performed on the dietary and fecal material included protein (Ramont et al. 1964), total lipid (Bligh and Dyer 1969), ash, HROM (Buddington 1980), HRA (Bowen 1981), and crude fibre (CF) content (Buddington 1980). Standard techniques were adopted to analyze samples of 250–500 mg content, the details being summarized in Table 1.

Results and Discussion

Experiments on *Etroplus suratensis*

In these experiments, the dry matter, protein, and lipid digestibility of the macrophyte *Hydrilla verticellata*, fed to satiation to three different size groups of *E. suratensis*, was determined using indigenous markers, i.e., HROM, HRA, and CF. The food consumption, fecal output, and HROM, HRA, and CF in the food and fecal material are presented in Table 2.

It is evident from the table that the percentage recovery of HRA was variable, whereas that of HROM and CF did not differ significantly ($P>0.01$) from 100%. CF and HROM basically refer to the same group of materials, cellulose and chitin (when present) are the chief constituents of HROM, and cellulose and lignin are the major components of the CF fraction. HRA is composed of the mineral ash resistant to acid digestion.

Of these fractions, HRA is found in the least quantity in the macrophyte. The variability observed, however, could be a result of higher experimental error when compared with that related to HROM and CF.

The dry matter and protein digestibility estimated based on the above indigenous components as markers are presented in Table 3. As the recovery of HRA was higher (Table 2), the digestibility estimates using HRA were consistently higher than those based on HROM and CF. On the other hand, Van Dyke and Sutton (1977) reported that the cellulose fraction of macrophytes is sparsely digested. The assimilation of cellulose and HROM by different species remains controversial and contradictory; for example, cellulose digestion up to 1.47% in channel catfish has been reported and Bowen (1981) reported assimilation of a small fraction of HROM in the detrital aggregate by *Tilapia mossambica*. De Silva et al. (1984) reported that in the natural diet of *Sarotherodon mossambicus* (from nine artificially constructed lakes) ash is concentrated to the same extent as that of HROM, CF, and HRA and indicated the possibility of using ash (the quantification of which is technically simple) as an indigenous marker in such studies.

Table 1. Procedures for the determination of HRA, HROM, and CF.

Hydrolysis Resistant Ash (HRA)

Original sample → ignite at 550°C for 12 hours → over 250 mg of ash → add 25 mL (1–2.5 v/v HCl) → boil and filter well using ashless filter paper of known weight → add hot water until acid free → ignite at 550°C for 12 hours → weigh residue left over is HRA

$$\% \text{ HRA} = \frac{\text{weight of HRA}}{\text{weight of original sample}} \times 100$$

Hydrolysis Resistant Organic Matter (HROM)

Sample (at least 0.3 g) → + 15 mL 80% CH_3COOH + 1.5 mL conc. HNO_3 → boil gently for 20 min → + 70% ethanol → centrifuge → in sequence rinse with warm (a) 70% ethanol (b) benzene (c) petroleum ether → dry at 100°C → weigh (x) → ignite at 550°C for 12 hours → weigh (y)

$$\% \text{ HROM} = \frac{(x - y) \times 100}{\text{sample weight}}$$

Crude Fibre (CF)

Fat free sample (over 200 mg) (residue from Bligh and Dyer (1959) method could be utilized) → + 10 mL 5% H_2SO_4 + 20 mL distilled water → boil for 30 min maintaining constant volume of the mixture → filter → wash with boiling water → + 10 mL 5% NaOH → boil for 30 min → filter → wash with boiling water → wash with 1% HCl → wash with boiling water → wash with 95% alcohol → dry overnight at 100°C → weigh (x) → ignite at 550°C for 12 hours → weigh (y)

$$\% \text{ CF} = \frac{(x - y) \times 100}{\text{sample weight}}$$

Table 2. Food consumption, fecal output, and HROM, CF, and HRA in the food and fecal material (adopted from De Silva and Perera 1983).

Group	No.	Mean length (mm)	Mean weight (g)	Food consumption ($mg/g \cdot day^{-1}$)	Fecal output ($mg/g \cdot day^{-1}$)	Indigenous markers					
						In food			In feces		
						HROM	CF	HRA	HROM	CF	HRA
I	7	82±0.32 (77–84)	9.36±1.35 (8.15–13.97)	8.85±3.97 (1.25–16.52)	5.34±1.19 (3.61–7.39)	1.91	1.17	0.53	2.21 (116)	1.24 (106)	0.71 (134)
II	7	86±0.41 (78–91)	10.68±1.42 (8.18–12.66)	12.51±7.78 (1.31–26.59)	6.91±4.4 (2.04–15.4)	1.25	0.77	0.38	1.23 (98)	0.73 (95)	0.46 (121)
III	5	101±0.54 (95–110)	18.14±2.06 (7.43–21.03)	8.02±3.78 (1.15–18.83)	4.01±1.59 (1.65–7.61)	1.94	1.19	0.50	1.98 (102)	1.07 (91)	0.54 (108)

Notes: Ranges given in parentheses for length, weight, food consumption, and fecal output. Percentage recovery given in parentheses for indigenous markers in feces.

Table 3. Mean percentage dry matter, protein, and lipid digestibility of *H. verticellata* for the different experimental groups of *Etroplus suratensis* obtained using different inert markers (adopted from De Silva and Perera 1983).

	Group I			Group II			Group III			Mean of three groups			Grand mean
	HROM	CF	HRA	HROM	CF	HRA	HROM	CF	HRA	HROM	CF	HRA	
Dry matter	38.0^a	34.6^a	50.0^b	36.1^a	37.5^a	51.9^b	39.9^a	35.1^a	44.2^b	38.3^a	35.5^a	44.8^b	41.3
Protein	60.6^a	58.4^a	68.2^b	60.1^a	60.9^a	—	62.5^a	59.5^a	64.6^b	61.4^a	59.2^a	65.6^b	64.3
Lipid	64.4^a	62.4^a	71.2^b	68.9^b	69.5^b	—	67.0^a	64.3^a	68.8^b	65.5^a	65.0^a	70.1^b	67.2

Notes: For any one parameter, values followed by the same superscript are not significantly different at the 5% level.

Table 4. Overall mean percentage dry matter, apparent protein, and energy digestibility of *S. niloticus* fry maintained on four experimental diets (D1 – D4) at three salinities (adopted from De Silva and Perera 1984).

	Fresh water				5‰ salinity				10‰ salinity			
	D1	D2	D3	D4	D1	D2	D3	D4	D1	D2	D3	D4
Dry matter	67.0 (±5.1)	66.2 (±4.3)	59.0 (±7.1)	55.6 (±6.4)	67.9 (±2.4)	68.4 (±4.0)	65.2 (±6.3)	59.8 (±5.8)	68.6 (±3.3)	65.3 (±6.5)	64.0 (±6.6)	64.0 (±7.1)
Protein	74.9 (±6.9)	87.1 (±3.4)	84.5 (±2.8)	83.3 (±1.9)	71.7 (±6.2)	86.6 (±3.4)	86.6 (±1.9)	84.2 (±2.2)	72.9 (±4.9)	86.9 (±3.8)	85.6 (±2.7)	85.6 (±2.4)
Energy	75.0	74.8	77.4	75.1	73.6	75.9	80.8	77.6	76.0	76.3	83.0	83.9

Notes: Standard deviation given in parentheses. The dietary protein contents of the diets were 9.6, 22.0, 28.0, and 30.4% for diets D1, D2, D3, and D4 respectively.

A final comparison of the indigenous markers considered here indicates that the near 100% recovery of HROM in the feces and possible assimilation of CF by fish (Niederholzer and Hofer 1979) makes the former a more reliable marker.

Experiments on *Sarotherodon niloticus* Fry: Artificial Diets

The results of digestibility experiments carried out on *S. niloticus* fry (weight range 50–750 mg) at different salinities using Cr_2O_3 as a marker are summarized in Table 4. In these experiments, 3% Cr_2O_3 was used in the diets as the fecal material collected was small and to permit reliable estimations. This is a deviation from the levels normally used in digestibility studies.

It is evident that for any one diet, total dry matter, protein, and lipid digestibility were not significantly affected by salinity. As expected, however, the dry matter and protein digestibility varied with the dietary protein content.

General Comments

In both sets of experiments, it was noted that the dry matter and protein digestibility varied from day to day, indicative of a possible rhythmicity in digestibility (De Silva and Perera 1983, 1984). It is too early, however, to predict the validity of these observations in aquacultural practices. Further experimentation is needed, therefore, to clarify these observations.

In light of the observations presented earlier and also bearing in mind the technical errors that could arise in Cr_2O_3 estimations and the physical risks involved, it would be useful to consider the use of markers such as HROM and CF in digestibility studies even when artificial diets are being utilized.

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Protein Requirements of Catfish Fry, *Pangasius sutchi*, Fowler

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Seven experimental diets containing 20, 25, 30, 35, 40 , 45, and 50% protein were fed to catfish fry contained in 21 circular concrete tanks (350 L) for 60 days. Each dietary treatment was randomly assigned to three tanks, each stocked with 100 fish. The fish were fed 7 days a week at a rate of 10% of fish body weight and their daily feed allowances were increased weekly on the basis of weight gain. Growth, survival rates, and feed conversion ratios for each diet were compared. Statistical analyses indicated that, of the diets tested, a diet containing 25% protein produced optimum growth.

Introduction

It has been established that protein is required by all animals for body maintenance and growth, and that the protein level needed for these functions varies with the species and culture environment (Munson et al. 1954; Phillips et al. 1957; Delong et al. 1958; Lovell 1972). For fish, the optimum amount of protein in formulated feeds is important because either low or high levels of protein may lead to poor growth. As well, excess protein in fish diets may be wasteful and cause the diets to be unnecessarily expensive.

The dietary protein level needed by catfish for optimum growth has been demonstrated in recent years by many investigators. Thus far, the majority of the work conducted has been carried out on American catfish (Nail and Shell 1962; Hastings 1969; Hastings and Dupree 1969). There has been virtually no nutritional work carried out using Thai catfish reported in the literature.

This study, therefore, was designed to provide information on the level of dietary protein needed by catfish fry for optimum growth in a laboratory-type culture where natural food is limited. Specific objectives included determining growth and survival rates as well as feed conversion ratios for fish grown from fry to fingerlings using nutritionally complete diets containing varying levels of protein.

Materials and Methods

Twenty-one circular concrete tanks, each measuring 100 cm in diameter and 75 cm in height, at the Department of Aquaculture hatchery house,

Kasetsart University, Bangkhen, Bangkok, were used in the experiment. The 21 tanks were each filled with 350 L of tap water that was stored for at least one night before being used. On 3 September 1979, the tanks were each stocked with 100 catfish fry averaging 0.2 g body weight. The tanks were randomly arranged into seven feeding treatments with three replications.

Seven diets containing 20, 25, 30, 35, 40, 45, and 50% crude protein were used in the experiment. To minimize changes in protein quality as the protein level varied, a nearly constant ratio of one part animal protein to three parts plant protein was maintained for all seven diets. The composition of the experimental diets is given in Table 1. The diets were processed as paste-form, sinking feeds as described by Chuapoehuk and Pothisoong (1978).

The fish were fed twice daily (0900 and 1500 hours) 7 days a week at a rate of 10% of fish body weight (percentage moisture) throughout the experimental period. Every week, a minimum of 40 fish were removed, using a net, from each tank and weighed to determine their average weight gains. Daily feed allowances were adjusted weekly based on the average weights of the fish in each treatment.

After weighing the fish, each tank was cleaned to prevent accumulation of fecal materials and reduce algal growth to a minimum. The same source and amount of water were then used to refill the tanks before the weighed fish were returned to their respective tanks.

On 2 November 1979, the final weight of the fish in each tank was measured and a final count was made to determine the overall weight gain and survival rate. The feed conversion for each tank was calculated as the ratio of the amount of feed given to the amount of weight gained. Average weight gain, survival rate, and feed conversion ratio for each of the treatments were estimated and compared statistically.

Results and Discussion

Average weight gains, feed conversion (feed : gain) ratios, and survival rates of the experimental fish are presented in Table 2. The greatest growth

Table 1. Percentage composition of experimental diets.

Ingredient	Dietary crude protein (%)						
	20	25	30	35	40	45	50
Fish meal	17	21	25	28	33	38	41
Soybean meal	23	28	34	41	45	50	56
Broken rice	52	44	35	26	18	9	1
Animal fat	6	5	4	3	2	1	—
Di-calcium phosphate	1	1	1	1	1	1	1
Salt	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Vitamin premix[a]	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Trace mineral premix[b]	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Antibiotic[c]	0.05	0.05	0.05	0.05	0.05	0.05	0.05

[a] Contains vitamin A, C, and D_3 in amounts equivalent to those recommended by the National Research Council (1976) for a complete ration for warm-water fish.

[b] Includes $CaCO_3$, $MnSO_4 \cdot H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, and $FeSO_4 \cdot 7H_2O$. The proportions used are the same as those recommended by the National Research Council (1976) for a practical diet for warm-water fish.

[c] Oxytetracycline.

Table 2. Average weight gain, feed conversion, and survival rate for catfish fry fed the experimental diets.

Diet (% protein)	Tank	Average weight gain per fish (g)	Feed conversion	Survival rate
20	12	2.540	1.46	99.0
	16	2.680	1.49	82.0
	19	2.640	1.40	99.0
		(2.620[a])	(1.45[a])	(93.3[a])
25	5	3.050	1.40	99.0
	20	2.500	1.42	100.0
	23	2.830	1.30	100.0
		(2.793[b])	(1.37[a])	(99.7[a])
30	4	3.000	1.27	100.0
	13	2.650	1.34	93.0
	14	2.900	1.30	98.0
		(2.850[b])	(1.30[a])	(97.0[a])
35	2	2.830	1.30	100.0
	8	3.300	1.25	98.0
	17	2.500	1.36	100.0
		(2.877[b])	(1.30[a])	(99.3[a])
40	6	2.800	1.39	98.0
	10	2.600	1.48	98.0
	18	2.550	1.48	99.0
		(2.650[a])	(1.45[a])	(98.3[a])
45	3	2.300	1.75	89.0
	21	2.450	1.86	76.0
	22	2.180	1.88	70.0
		(2.410[c])	(1.83[b])	(78.3[b])
50	7	2.000	2.28	68.0
	9	2.300	2.00	75.0
	15	2.050	2.15	75.0
		(2.117[d])	(2.14[c])	(72.7[b])

Note: Average values in parentheses. Averages followed by common superscripts are not different at the 0.05 probability level.

took place in those fish receiving the diets containing 25, 30, and 35% protein. The lowest protein diet (20%) and the diet containing 40% protein provided similar rates of growth; these rates being significantly greater than those attained with the 45% protein diet. The highest protein diet (50%) produced significantly less growth than any of the other experimental diets.

Feed conversion ratios were lowest for the 30 and 35% protein diets. These conversion ratios were not significantly less than those of the diets containing 20, 25, and 40% protein but were statistically lower than that of the 45% protein diet. The 50% protein diet was significantly less efficiently converted into weight gain than any of the other diets.

Survival rate for the 25% protein diet was not statistically higher than that of the 20, 30, 35, and 40% protein diets, but was significantly greater than that of the diets containing 45 and 50% protein. The difference in survival rate between the 45 and 50% protein diets was not large but the 50% protein diet had the poorest survival rate.

Weight gain data indicated that under the feeding regime followed in this study there was no significant growth advantage in increasing the dietary protein level above 25%. Also, survival rates were highest for the 25% protein diet. Feed conversion was nearly as efficient for the 25% protein level as the three higher protein levels.

Conclusions

Based on the data collected during this study and within the confines of the conditions under which the study was carried out, it can be concluded that, of the levels of protein tested, a minimum level of 25% protein is needed in the diet for optimum growth of catfish fry in laboratory or intensive cultures. If feeding is carried out under natural environmental conditions, it may be possible to justify lower levels of protein in the diet.

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The Effect of Three Diets with Variable Protein Levels on Ovary Development and Fecundity in *Leptobarbus hoevenii*

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The effect of three diets with variable protein levels of 24, 32, and 40% crude protein on the performance of *Leptobarbus hoevenii* broodstock was studied. In terms of body weight, the fish fed the 24% diet were significantly smaller ($P < 0.05$) compared with those fish fed the other two diets. Fish fed diets with higher protein levels (32 and 40% crude protein) produced significantly larger ovaries than those fed diets with 24% crude protein. Gonadosomatic index (GSI) values were also significantly higher ($P < 0.05$) with the 32 and 40% diets than with the 24% diet. There were no significant differences in the average weight of the individual egg and in the number of eggs per gram of ovary tissue among all three treatments. Fecundity was significantly higher ($P < 0.05$) in fish fed 32 and 40% crude protein diets than in those fed the 24% crude protein diet.

Introduction

The development of the ovary in any potential broodstock depends to a large extent on the age, size, nutritional status, and individual physiology of the fish, as well as the environmental factors influencing it. A good account of the extrinsic factors affecting the development of the ovary and final spawning in potential breeders is provided by Vlaming (1974). The nutritional status of the fish encompasses both the availability of the feed in suitable amounts and also the quality of the feed, i.e., food possessing all the essential nutrients such as protein, lipids, energy, vitamins, and minerals.

Poynter (1976) clearly demonstrated that the fecundity of hatchery-reared lake trout appears to be directly related to food availability. Fish fed at a daily rate of 0.75% of their body weight produced more and larger eggs with higher fertility than those fed at a rate of 0.5% of their body weight. Scott (1962) found that variations in egg number are related to the size of the fish, size of the eggs, and adequacy of the diet. Hester (1964), working with *Lebistes reticulatus*, determined that reduced numbers of offspring and reduced large- and middle-sized oocyte counts were associated with reduced rations. Bagenal (1969) also observed that the number of eggs produced by the brown trout *Salmo trutta* was higher in the better fed fish. Dahlgren (1980), who studied the effect of three different dietary protein

levels on the fecundity of the guppy *Poecilia reticulata*, concluded the following: the average gonadosomatic index (GSI) was highest in groups fed the highest protein diet; fecundity of the fish was not affected by diet; and higher body weight was found in fish fed higher protein diets. A similar pattern of increments of dietary protein levels resulting in a higher growth rate in channel catfish, *Ictalurus punctatus*, was determined by Nail (1962), Shell (1963), and Tiemeier et al. (1965). The influence of dietary protein on growth rate was also demonstrated in fingerling rainbow trout, *Salmo gairdneri* (Zeitoun et al. 1976).

The aim of this paper, therefore, is to observe the effect of dietary protein levels on ovary development in *Leptobarbus hoevenii* and its influence on the fecundity of the fish.

Materials and Methods

Potential broodstock of unknown age were collected at random from a number of holding ponds and stocked in experimental ponds after weighing. Sick fish, affected by external parasites, and those that were deformed were discarded. The fish were stocked at a rate of 250 fish per 0.44 ha, with two replications per treatment. At this stage of stocking, it was not possible to sex the fish by external observation and it was assumed that 50% of the fish were females.

The three treatments/diets with variable protein levels are presented in Table 1. Throughout the experiment, there were variations in the level of the ingredients (mainly fish meal) in the diet due to variations in the nutritional composition of the ingredients received; however, the total crude protein of each experimental diet was maintained at 24, 32, and 40% respectively. The fish were fed twice daily at a rate of 1% of their total body weight. Sampling was carried out at monthly intervals to determine the increase in body weight to facilitate adjustment in the feeding regime.

At the time of sampling, fish were observed for development of the belly to indicate if any ovary development was taking place, as a sign of external sexual maturity in the females. Male sexual maturity was determined by their ability to produce semen. To facilitate the accurate determination of the swelling of the belly, the fish were starved for a day prior to sampling. Potential breeders were later collected for induced breeding, first by

Table 1. Composition and nutrient content of the experimental diets.

Ingredient (%)	Diet		
	M1	M2	M3
Fish meal	25.00	37.00	55.75
Soybean	18.00	29.00	27.00
Copra cake	14.00	10.00	7.00
Maize	20.00	10.00	3.00
Rice bran	16.75	7.75	1.00
Tapioca	2.00	2.00	2.00
Vitamin mixture[a]	0.60	0.60	0.60
Mineral mixture[a]	3.65	3.65	3.65
Calculated crude protein (%)	24.00	32.00	40.00
Analyzed crude protein (%)	23.40	30.20	38.30

[a] As recommended in National Research Council (1977).

external selection, then by cannulation to confirm that egg formation had taken place in all three treatments. Following this confirmation, 20 potential breeders were selected from each replication by an experienced breeder based totally on external features. The fish were then sacrificed for measurements of the following parameters: standard length, total body weight, weight of ovary, and samples of the ovary for measurements of egg weight and absolute fecundity. Samples of the ovary were taken from the anterior, middle, and posterior parts of the ovary, weighed and placed in Gibson's fluid for later counting.

Results and Discussion

Selection of Breeders

Leptobarbus hoevenii are found mainly in the large rivers of northeastern Malay Peninsula. The breeding season for this fish has been dictated mainly by the distinct rainy season (Tan 1980). Spawning may be triggered by the punctual swelling of the rivers, followed by flooding of the surrounding areas.

Effective external stimuli such as these are completely lacking in static shallow ponds and monthly samplings have indicated that breeders can be obtained throughout the year. Although breeders were found year round, their belly development was very small compared with other species of breeders such as Bighead (*Aristichthys nobilis*), grass carp (*Ctenopharyngodon idella*), and lampam java (*Puntius goinonotus*). This problem is clearly emphasized by the fact that some of the breeders selected by external observation were, in fact, females in a very early stage of egg development or immature males having good body confirmation.

The lowest breeder selection error of 7.5% was attained with fish fed the 24% crude protein diet, followed by fish fed the 40% crude protein diet with a selection error of 10%. The highest selection error of 25% was attained with fish fed the 32% crude protein diet. This diet was earlier identified as being the most suitable for the growth of *Leptobarbus hoevenii* and was classified as a production diet (Pathmasothy and Omar 1982). Thus, this diet may have promoted good body development resulting in the eclipse of the belly development and leading to greater selection error.

Standard Length and Total Body Weight

The average standard length of the fish fed the 24% crude protein diet, with a value of 42.5 cm, was significantly smaller ($P < 0.05$) than that of fish fed the other two treatments. No significant difference was found between the standard lengths of the fish fed the 32 and 40% crude protein diets, with values of 44.4 and 44.0 cm respectively. In terms of total body weight, however, fish fed the 32% crude protein diet had a significantly higher ($P < 0.05$) body weight (2624 g) than those fed the 24 and 40% crude protein diets (2151 and 2321 g respectively). There was no significant difference, however, between the latter two.

The relationship between the standard length and total body weight of the females is presented by the following linear regression equations:

24% crude protein diet	$Y = 171.86X - 5150.18$	$r^2 = 0.66$
32% crude protein diet	$Y = 147.07X - 3912.14$	$r^2 = 0.81$
40% crude protein diet	$Y = 122.41X - 3068.29$	$r^2 = 0.32$

It can be observed that the best correlation between standard length and body weight ($r^2 = 0.81$) was obtained from fish fed the 32% crude protein diet. In most normal, well-fed populations, the relationship between standard length and total body weight is, in fact, represented by a high degree of correlation. Any deviations found may be attributed to either scarcity of food or uneven development of the ovary, whose uneven increase in weight may have interfered in the harmonious relationship.

Weight of Ovary and GSI Values

The average weights of 158.61 and 156.92 g attained in fish fed the 32 and 40% crude protein diets, respectively, were significantly higher ($P < 0.01$) than that attained in fish fed the 24% crude protein diet, with a value of 96.40 g. Although there was no significant difference in the average ovary weight of fish fed the 32 and 40% crude protein diets; in general, higher individual ovary weights were found in those fish fed the 40% crude protein diet when compared on the basis of common standard lengths.

The relationships between standard length and ovary weight for the three treatments are as follows:

24% crude protein diet	$Y = 16.53X - 606.05$	$r^2 = 0.40$
32% crude protein diet	$Y = 22.05X - 821.20$	$r^2 = 0.46$
40% crude protein diet	$Y = 31.83X - 1244.50$	$r^2 = 0.35$

Thus, for fish of a given standard length, those fed higher protein diets tended to produce larger ovaries. This was further supported by the fact that the GSI values were higher in fish fed higher protein diets. Fish fed the 40% crude protein diet produced the highest average GSI value of 6.69, which was significantly higher ($P < 0.05$) than the GSI value of fish fed the 24% crude protein diet (average value of 4.17), which was the lowest. The average GSI value of 5.84, attained from feeding the 32% crude protein diet, although not significantly different from the 40% treatment, was significantly greater ($P < 0.05$) than that obtained using the 24% crude protein diet.

Weight of Egg and Number of Eggs per Gram

There was no significant difference in the average weight of the individual egg from the three treatments; however, fish fed the 40% crude protein diet did produce larger eggs, with an average weight of 1.3006 mg, followed by those fed the 24 and 32% crude protein diets, with weights of 1.278 and 1.267 mg respectively. It can be concluded, therefore, that the amount of protein in the diet did not influence the weight of the eggs. A similar conclusion was drawn by Scott (1962), working with rainbow trout, *Salmo gairdneri*. Dahlgren (1980), working with *Poecilia reticulata*, noted that the ovum size remained the same between groups fed diets with different protein levels. A general observation noted from all three treatments was that as the GSI and weight of the ovary increased there was a decrease in the weight of the individual egg.

In terms of the number of eggs per gram of ovary tissue, there was no significant difference among the three treatments. Fish fed the 32% crude protein diet produced 807 eggs per gram, followed by the diet with 24% crude protein (792 eggs per gram) and the 40% crude protein diet (790 eggs

per gram). It may be suggested that the nutritional status of the fish will not influence the number of eggs per gram of ovary tissue or the weight of the egg but that something other than diet predetermines them.

Fecundity

Fish fed the 32% crude protein diet, which produced an average of 134 563 eggs per female, had the highest fecundity value but it was not significantly different from that of those fish fed the 40% crude protein diet (130 035 eggs per female). Both of these values, however, were significantly greater ($P < 0.05$) than that obtained with the 24% crude protein diet, which resulted in the production of an average of only 82 680 eggs per female. It can be concluded, therefore, that higher protein diets influence the total number of eggs produced.

Valuable protein must be absorbed by the fish for accumulation during egg formation. Dawson and Grimm (1980), working with *Pleuronectes platessa*, determined that in winter, when the plaice do not feed but grow large ovaries, 40% of the protein stored in the body is utilized, of which 33% is devoted to egg formation. In tropical situations where feeding takes place year round, it has been determined that the protein level remains fairly constant (Hails 1983), indicating the natural feed's ability to supply the necessary protein for egg formation.

The major factor affecting fecundity in most fish appears to be the availability of feed. Adequacy of diet was attributed to variations in the total number of eggs in *Salmo gairdneri* (Scott 1962). Bagenal (1969) concluded that more of the better-fed fish were mature, grew faster, and contained significantly more eggs. The fecundity of hatchery-reared trout appears to be directly related to food availability (Poynter 1976). The adequacy of the feed, therefore, may be suggested as being a broad prerequisite for the availability of all essential nutrients in the diet.

The level of protein, in particular, may be one of the crucial factors. However, Dahlgren (1980), working with *Poecilia reticulata*, found no significant difference in the fecundity of the females fed diets containing 47 and 31% protein or between females fed diets containing 47 and 15% protein, but observed a higher number of ova per fish in females fed the 31% protein diet than those fed the 15% protein diet. This contradiction may be attributed to the different reproductive system of the guppy, which is a viviparous fish.

In general, it can be concluded that fecundity can be influenced by the level of protein in the diet available to the female fish up to a certain critical level, beyond which excess protein may have no positive effect on fecundity. The critical protein level may be influenced by age, size, and the species involved. Female breeders with potential for further growth will, therefore, require more protein for body weight gain, maintenance, and egg formation. Old breeders that have reached optimal growth, as dictated by their genetic composition, may require less protein, which would be used mainly for maintenance and egg formation. The significantly low fecundity found in fish fed the 24% crude protein diet may be due to the fact that the level of protein supplied to these first-time breeders might not have been sufficient for growth, maintenance, and egg formation compared with that achieved with the 32 and 40% crude protein diets. However, the parameters measured to evaluate the diets may not be sufficient; therefore, additional parameters should be considered in future studies.

Acknowledgments

The author would like to acknowledge the Director General, MARDI, and the International Development Research Centre (IDRC) for their support of this project. The technical assistance of Fauzi bin Md. Dawan is also gratefully acknowledged.

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Cage Culture of *S. niloticus* in Sri Lanka: Effect of Stocking Density and Dietary Crude Protein Levels on Growth

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S. niloticus was cultured in cages (rearing volume 5 m^3) to evaluate the technical and economic feasibility of such activities. The fish were stocked in cages at three stocking densities (400, 600, and 800 fish/m^3) and were fed four diets of varying dietary crude protein levels (29, 25, 20, and 19%) for a period of 4 months. In all, 24 cages were used in the trials. The fish were fed 3% by body weight pellet feeds six times daily, commencing at 0800 hours. It was observed from the results that there was no significant difference in live weight gains (WG) and feed-conversion ratios (FCR) between dietary crude protein levels for each stocking density or between stocking densities for a particular dietary crude protein level ($P>0.05$). The protein digestibilities decreased from diet 1 (crude protein 29.1%) to diet 3 (crude protein 20%), in which rice bran was used in increasing amounts in the diet formulation. In diet 4 (crude protein 19%), which contained no rice bran, protein digestibilities were comparatively higher.

Introduction

Preliminary investigations of cage culture of *S. niloticus*, conducted between 1980 and 1982 (Wannigama and Weerakoon 1982; Anonymous 1982), indicated clearly the technical feasibility of this method of culture in Sri Lanka. The work reviewed here deals with experimental studies aimed at maximizing *S. niloticus* yield from cage culture operations by elucidating on the optimum stocking density. The studies also deal with the response of *S. niloticus* to diets of varying protein content with a view toward minimizing feed-cost inputs.

Materials and Methods

Cage-Culture Trials

The site selected for the experiments was Udawalawe reservoir. Twenty-four cages (2.0 m × 2.0 m × 1.25 m) were utilized for the experiments. The design of the cage is described in Wannigama and Weerakoon (1982). The

cage frame was made of whole bamboo and the netting was 6-ply nylon of 1-cm mesh. The volume of the net cage under water was 5 m^3.

Three stocking densities (400, 600, and 800/m^3) were used in the experiments. Sampling of fish was carried out every 5th week and a random sample of 10% was measured for total length and weight. The initial body weight of *S. niloticus* in this study varied between 22 and 30 g/fish.

Four diets (Table 1) with different crude protein levels (19, 20, 25, and 29%) were fed to each group of fish at amounts equal to 3% by body weight six times a day for a period of 4 months. The feeds were given in pelleted form broadcast by hand. Wastage was minimal.

Digestibility Experiments

Digestibility of the experimental feeds was determined using chromic oxide as the marker (Furukawa and Tsukahara 1966). For these experiments, the initial size of *S. niloticus* used in the cage culture experiments was utilized and the experiments were conducted in 12 140-L circular fibreglass tanks incorporated with a flow-through system. Precautions suggested by Cho et al. (1982) were adhered to. Protein digestibility determinations were based on fecal material accumulated throughout the night. The experimental fish were fed four times a day commencing at 0800 hours. After the last feeding, the tanks were cleaned thoroughly of any unconsumed feed and replenished with clean, fresh water. The feces accumulated overnight were dried in an oven at 80°C for 24 hours. The dried feces, pooled over 5-day periods for each experimental group, were utilized for protein and chromic oxide analyses. Protein digestibility was determined by the biuret method, as modified by Raymont et al. (1964), on 80–100 mg aliquots of ground fecal material. Bovine serum albumen was used as the standard.

Results

Live Weight Gain

Percentage live weight gain (LWG) is expressed by the equation

$$\frac{W_t - W_o}{W_o} \times 100$$

where: W_o = initial weight
W_t = weight at time t days

Values for the grow-out period for each group of fish are given in Table 2. The variation in the percentage LWG between dietary crude protein levels

Table 1. Percentage composition of diets used for the cage-culture trials and dietary crude protein levels.

Ingredient	Diet no.			
	1	2	3	4
Fish meal	30	20	10	05
Chick mash	35	40	45	92
Rice bran	32	37	42	—
Shark-liver oil	03	03	03	03
Total	100	100	100	100
Crude protein	29	25	20	19

Table 2. Percentage live weight gain (LWG) and feed-conversion ratio (FCR) of *S. niloticus* fed four dietary crude protein levels at three stocking densities.

Diet no.	Protein (%)	Stocking density 400 fish/m³ %LWG	FCR	600 fish/m³ %LWG	FCR	800 fish/m³ %LWG	FCR
1	29	230	2.0	220	2.2	210	2.1
2	25	180	2.6	170	2.5	170	2.4
3	20	270	2.1	210	2.0	200	2.4
4	19	260	2.1	210	2.2	150	2.5

for each stocking density, as well as between stocking densities for a particular dietary crude protein level, was low and not significant ($P > 0.05$).

Table 3 presents the final mean weight and length of *S. niloticus* at each stocking density. It can be seen that variations in the final mean weight between dietary crude protein levels at a particular stocking density did not differ significantly ($P > 0.05$).

Feed-Conversion Ratio (FCR)

Table 2 lists the feed-conversion ratios for the grow-out period for each group of fish. As in the case of LWG, the variation in the feed-conversion ratios between dietary crude protein levels for each stocking density and between stocking densities for a particular dietary crude protein level was not significant ($P > 0.05$).

Digestibility of Diets

The protein digestibility values for the four diets are given in Table 4. The protein digestibility decreased with decreasing dietary crude potein levels except in the case of diet 4, which had a crude protein level of 19%. This may have been a result of the fact that the diet with 19% crude protein was commercial chick starter feed, with 5% fish meal and no rice bran. Therefore, the decrease in protein digestibility may have been a result of the presence of rice bran, which was of poor quality and was used in increasing amounts in diets 1 to 3. This was further substantiated by the presence of pieces of undigested rice husks in varying proportions in the fecal matter.

Discussion

Previous cage-culture trials carried out between 1980 and 1982 with *S. niloticus* using three stocking densities (100, 150, and 250/m³) and maintained on a diet of 22.5% crude protein content indicated that the stocking density could be increased beyond 250 fish/m³ (Wannigama and Weerakoon 1982; Anonymous 1982).

From the data presented, it has been shown that dietary crude protein levels and stocking densities did not have any influence on LWG of *S. niloticus*. This indicates further that the stocking density could be increased beyond 800 fish/m³.

There was no apparent difference in FCR at dietary crude protein levels of 19, 25, and 29%, although there was a slight increase in FCR at a dietary crude protein level of 20% at all three stocking densities. This increase, however, was not significant. Further experimentation is necessary to firmly establish that, for *S. niloticus* of mean weight 20– 30 g and at the stocking

Table 3. Mean length (cm) and weight (g) relationships of *S. niloticus*.

Diet no.	Protein level (%)	Stocking density: 400 fish/m³				600 fish/m³				800 fish/m³			
		Length		Weight		Length		Weight		Length		Weight	
1	19	17.0 (±1.82)	16.8 (±1.73)	92.2 (±31.7)	99.2 (±31.7)	14.6 (±1.76)	14.9 (±1.78)	66.4 (±20.0)	66.1 (±22.2)	16.0 (±1.89)	15.6 (±1.9)	86.1 (±30.9)	78.5 (±29.8)
2	20	16.2 (±1.92)	15.8 (±1.78)	84.4 (±27.3)	77.3 (±25.2)	14.9 (±1.86)	14.8 (±1.73)	70.9 (±26.0)	64.8 (±22.3)	15.9 (±2.03)	15.6 (±1.81)	80.6 (±30.0)	78.7 (±25.5)
3	25	16.1 (±1.86)	15.7 (±1.78)	85.4 (±31.2)	80.3 (±31.9)	16.0 (±2.58)	15.2 (±2.2)	84.8 (±37.8)	75.4 (±28.8)	15.2 (±1.5)	14.5 (±1.8)	67.1 (±20.0)	59.6 (±21.7)
4	29	16.6 (±1.69)	17.4 (±1.9)	93.5 (±26.6)	107.4 (±32.4)	14.9 (±1.71)	15.7 (±1.91)	65.9 (±20.9)	75.3 (±23.1)	14.9 (±1.9)	14.5 (±1.7)	67.9 (±27.2)	64.6 (±22.7)

Notes: Each experiment was carried out twice, thus the two columns of values. Standard deviation given in parentheses.

Table 4. Preliminary results of protein digestibilities (%) of the four diets used in the experiments.

Diet no.	Rice bran content	Protein	
		Content	Digestibility
1	32	29	86.2
2	37	25	82.0
3	42	20	78.8
4	0	19	91.9

densities tested, a dietary crude protein level of between 19 and 25% would be sufficient to obtain maximum growth. Jauncey and Ross (1982) found that, for *S. niloticus* of 6– 30 g mean body weight, the fish fed a dietary protein level of 25% produced 85% of the maximum specific growth rate exhibited by the same fish fed a dietary protein level of 30%. It is conceivable, therefore, that *S. niloticus* above 30 g body weight would require less protein, as is indirectly evident from the present study.

The digestibility experiments were of a preliminary nature. Proper quality control of feed ingredients was not possible because of the large quantities of feed required and the fact that facilities for such quality control were not available. The quality of rice bran and fish meal, for example, varied from time to time from the same source and contained considerable quantities of silica particles. The rice bran used contained considerable quantities of rice husks. This may have been the cause of discrepancies in the protein digestibilities observed for diets 1 to 4. It is important, therefore, to consider the digestibility results presented in this paper as information only. Because of this fact, a proper conclusion could not be obtained with regard to digestibility.

Acknowledgments

I wish to extend my sincere thanks to Dr S.S. De Silva, Professor of Zoology and Dean of the Faculty of Science, for the valuable advice given during the preparation of this paper and also for the use of the laboratory facilities at Ruhuna University College, Matara, Sri Lanka.

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Water-Soluble Vitamins Essential for the Growth of *Clarias*

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Catfish fingerlings (*Clarias batrachus* Linn.) were fed eight test diets: diet 1, the control, was a complete vitamin diet; diets 2–8 were devoid of water-soluble vitamins B_1 (thiamine), B_2 (riboflavin), B_6 (pyridoxine), pantothenic acid, folic acid, niacin, and vitamin C (ascorbic acid) respectively. Average weight gains, growth rates, and the effect of the vitamin-deficient diets were then observed.

Introduction

The walking catfish (*Clarias batrachus* Linn.) is native to Southeast Asia, occurring in rivers, canals, lakes, swamps, and flooded marshes or fields. *Clarias* is one of the most economically important cultured species in Thailand. The fish can tolerate poor water quality, is adaptable to high stocking density, eats a wide range of supplemental feeds, and is able to spawn naturally in captivity. The problem that farmers are currently facing, however, is high mortality. This might be due to stress susceptibility induced by inbreeding or malnutrition.

The increasing importance of this species in commercial fisheries demands considerable research on its nutritional requirements. Water-soluble vitamins have been identified as being essential for salmon and trout (Halver 1957; Coates and Halver 1958; Kitamura et al. 1967; Phillips et al. 1955), channel catfish (Dupree 1966), carp (Aoe et al. 1967, 1969), and eels (Arai et al. 1972). No research on the vitamin requirements of *Clarias* has been reported. The present study, therefore, was designed to ascertain the specific water-soluble vitamin requirements of *Clarias* on the basis of growth and mortality.

Materials and Methods

Facilities and Fish

Thirty-two 50 cm × 90 cm × 50 cm glass aquaria, located in a wet laboratory at the National Inland Fisheries Institute, were used in a 24-week feeding experiment. The well water in each aquarium was aerated and changed every other day. *Clarias* fingerlings, obtained from a private hatchery in Chacheongsao Province, were used in the study. Initially, the fingerlings weighed 4–5 g, with an approximate length of 7–8 cm. They were stocked in August 1982 at a density of 12 fish per aquarium.

Test Diets

A semipurified complete diet was used as the control diet. The composition of the complete diet, including vitamins and minerals, is presented in Table 1. Thiamine, riboflavin, pyridoxine, pantothenic acid, folic acid, niacin, and ascorbic acid were individually deleted from the complete diet to form seven test diets.

The dry ingredients of each diet were mixed in a Hobart mixer for 6–7 min, then 6% oil and 30% water were added and mixed. The moist diet mixture was passed through a 1.6-mm diameter die in a food grinder. The spaghetti-like diet was then broken into 1.0–1.5 cm length pellets and kept in the freezer until it was fed to the fish.

Management

Fish from the farm were stocked in a fibreglass tank, checked, and treated for parasites and diseases. They were then trained to feed on pellets for 3 weeks. Prior to starting on the test diets, the fish were stocked in the aquaria and fed a complete diet for 1 week. The aquaria were randomly chosen to receive either the complete vitamin diet or one of the seven vitamin-deficient diets. Each diet was fed to four replicate aquaria. The fish were fed twice daily, 0900 hours and 1600 hours, 6 days per week. The feeding rate was 10% of the body weight per day. The fish were sampled triweekly for weight measurements and observed for gross signs of vitamin deficiency. The feed allowance was adjusted subsequent to weight measurement. The fish were fed the test diets for 24 weeks. At the end of 12 weeks, after vitamin-deficiency symptoms had developed, two of the four aquaria receiving vitamin-deficient diets began receiving the complete diet.

The fish were observed twice daily at feeding time and a close examination was made during each weighing period every 3 weeks. A gross postmortem examination was made of the liver, kidneys, stomach, and intestines. No histological examination was made. The growth data were subjected to analyses of variance (Steel and Torrie 1960).

Table 1. Composition of the complete vitamin test diet for *Clarias*.

Ingredient	Percentage composition
Casein, vitamin free	29.0
Corn dextrin	30.0
Salad oil	6.0
Vitamin mix[a]	2.0
Mineral mix[b]	5.5
Cellulose	18.5
Carboxymethylcellulose	3.0
Gelatin	6.0

[a] Vitamin mix (mg/kg diet): vitamin A, 3; vitamin D, 0.05; vitamin E, 50; vitamin K, 10; choline, 550; niacin, 100; riboflavin, 20; pyridoxine, 20; thiamine, 20; D-calcium pantothenate, 50; biotin, 0.1; folic acid, 5; vitamin B_{12}, 0.002; vitamin C, 100; inositol, 100.

[b] Mineral mix (g/kg diet): $CaHPO_4 \cdot 2H_2O$, 2.07; $CaCO_3$, 1.47; KH_2PO_4, 1.0; NaCl, 0.6; $MnSO_4 \cdot H_2O$, 0.035; $FeSO_4 \cdot 7H_2O$, 0.05; $MgSO_4$, 0.30; KIO_3, 0.001; $CuSO_4 \cdot 5H_2O$, 0.003; $ZnCO_3$, 0.015; $CoCl_2$, 0.00017; $NaMoO_4 \cdot 2H_2O$, 0.00083; Na_2SeO_3, 0.00002.

Results and Discussion

Complete Vitamin Diet

Clarias receiving the complete diet exhibited weight gains averaging from 4.70 to 36.75 g during the first 12 weeks and 34.82 to 62.6 g during the second 12 weeks. Abnormality in morphology and behaviour was not observed, nor was any mortality during the 24-week period.

Thiamine-Deficient Diet

The results of feeding thiamine-deficient and complete vitamin diets are illustrated in Fig. 1. No significant differences in weight gain or mortality were demonstrated, nor did postperiod examination of the fish show any abnormal condition in the liver, kidneys, stomach, or intestines. The only abnormality observed in the fish was a dark skin colour.

Riboflavin-Deficient Diet

Results illustrated in Fig. 2 show no significant difference in average weight gain. The differences in cumulative mortality between the riboflavin-deficient group and the complete vitamin group at the end of 12 weeks and 24 weeks were highly significant. In fish fed the riboflavin-deficient diet, deaths began to occur after 5 weeks, followed by a reduction in food consumption and activity. Food consumption resumed, however, by week 12.

Examination of the riboflavin-deficient fish revealed fragile fins; hemorrhaging under the skin, at the fins, and around the eyes; eroded barbels; edema; fading of body colour; poor appetite; lethargy; pale gills; pale liver; and slight opaque lens at the end of the experiment.

The recovery test initiated after 12 weeks of feeding on the B_2-deficient diet indicated a significant decrease in mortality and improvement in deficiency symptoms and growth.

Pyridoxine-Deficient Diet

The results of utilizing this diet are illustrated in Fig. 3. The diet significantly retarded growth after 3 weeks. Death and deficiency symptoms developed after 8 weeks. The group showed mortality rates of 38.8 and 88.8% at 12 and 24 weeks, respectively, which was highly significant compared with the group fed the complete diet.

B_6-deficiency symptoms in *Clarias* involved eroded barbels, tetany, nervous disorders, loss of equilibrium, and erratic swimming habits such as whirling, twisting, and swimming in circles. Prior to dying, the fish surfaced frequently, floating on the surface or sometimes sinking to the bottom immediately, where they lay still and experienced rapid breathing. Gross postmortem examinations revealed eroded fins and lower jaw. The internal organs, however, including the stomach, liver, kidneys, intestine, and gill, appeared normal. Mortality and deficiency symptoms were eliminated after feeding on the complete diet for 3 weeks.

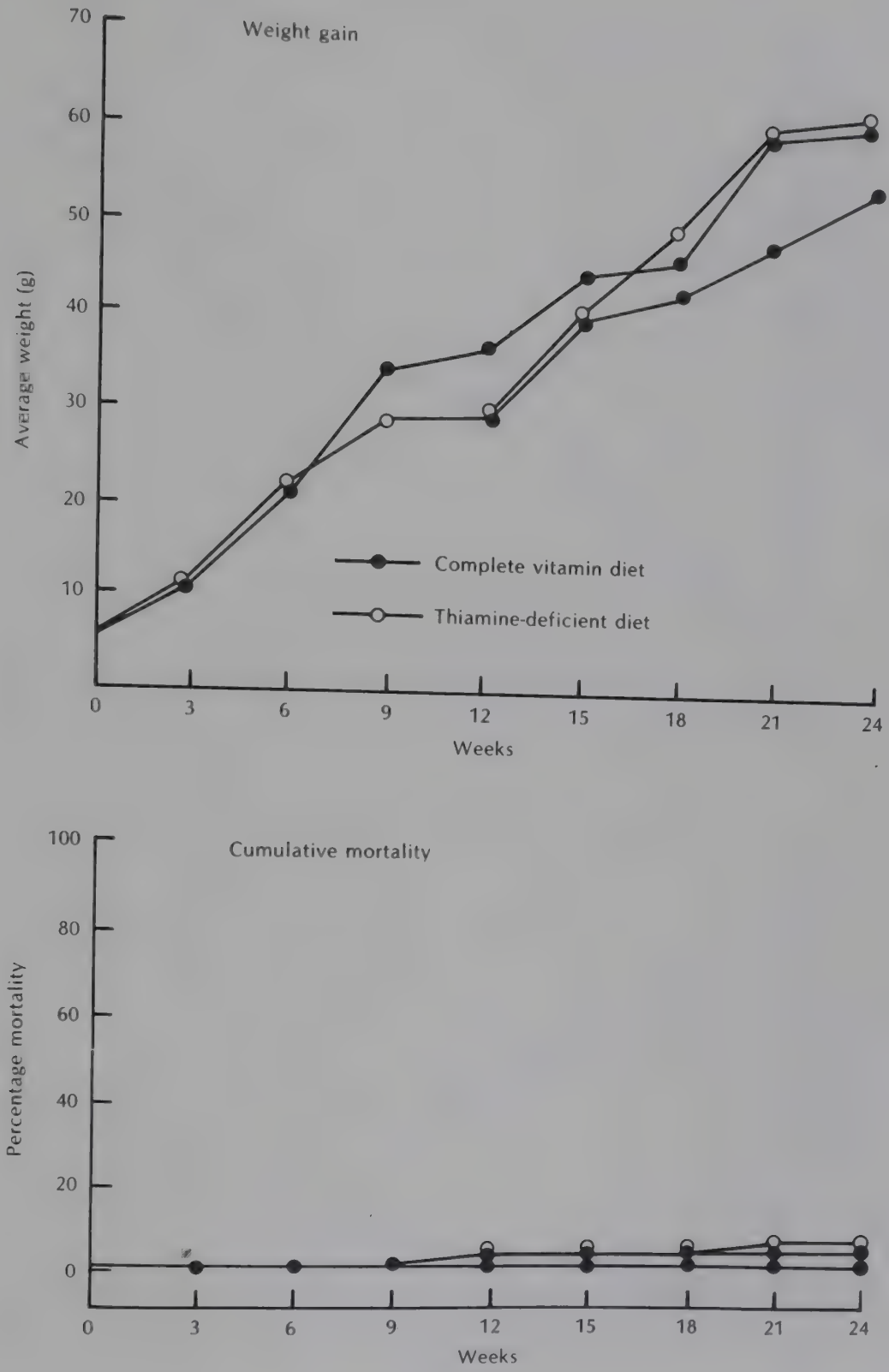


Fig. 1. Comparison of the average weight gains and cumulative mortalities of Clarias *fed thiamine-deficient and complete vitamin diets.*

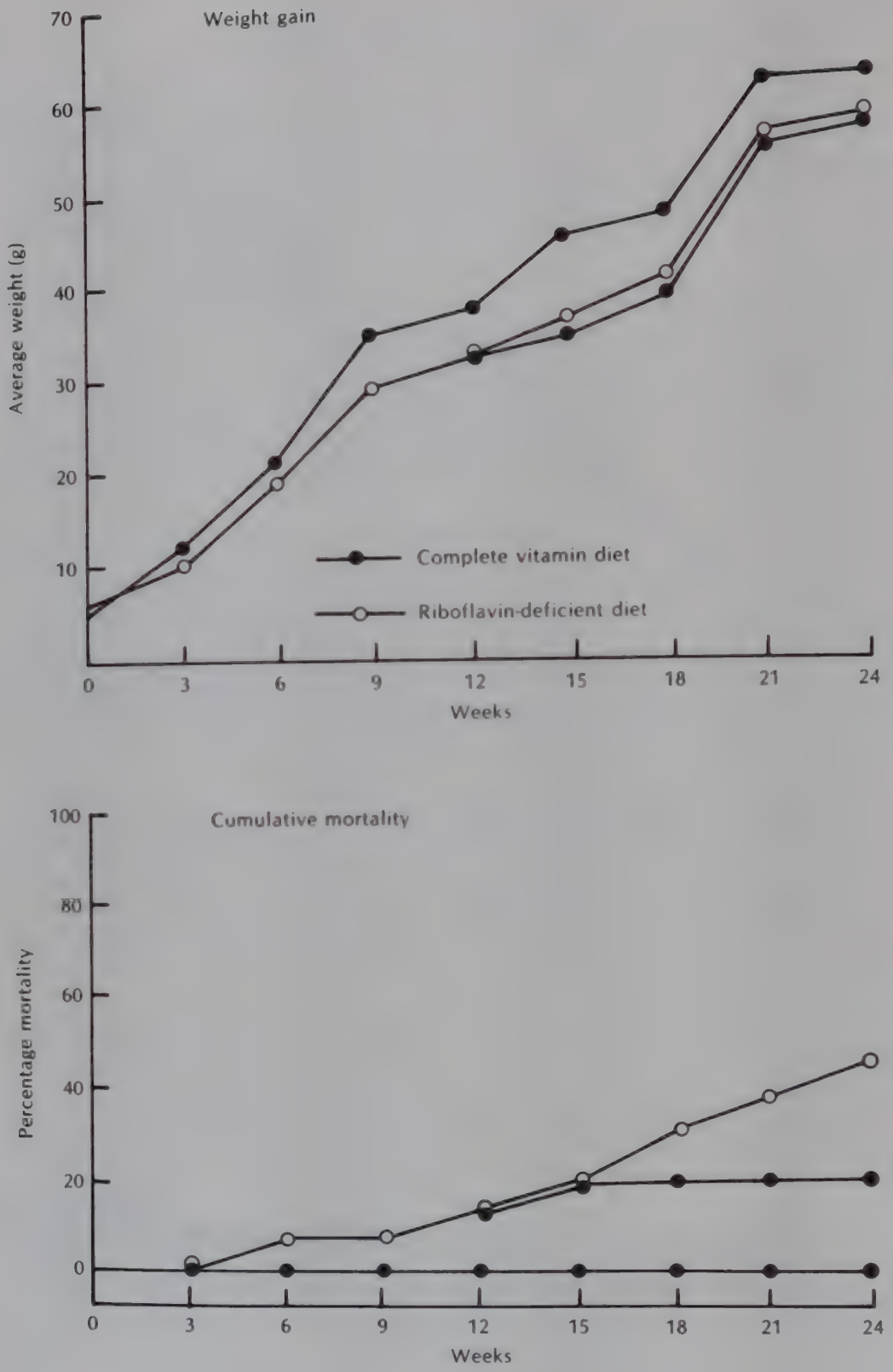


Fig. 2. Comparison of the average weight gains and cumulative mortalities of Clarias *fed riboflavin-deficient and complete vitamin diets.*

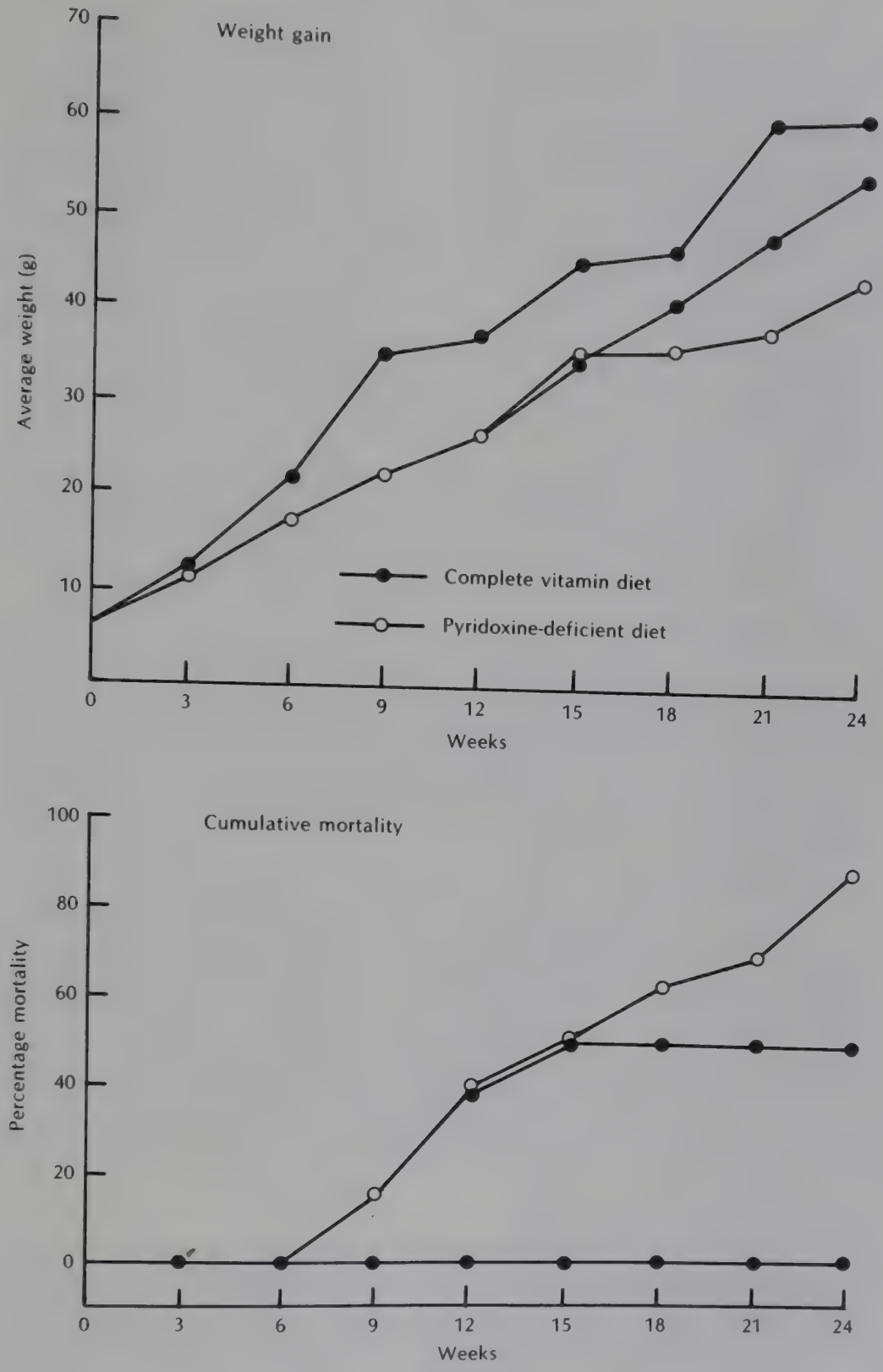


Fig. 3. Comparison of average weight gains and cumulative mortalities of Clarias *fed pyridoxine-deficient and complete vitamin diets.*

Pantothenic Acid Deficient Diet

Use of this diet resulted in significant differences in average weight gain after only 3 weeks of feeding (Fig. 4). There was an extreme reduction in food intake, accompanied by decreased activity and weight gain during weeks 9–12. Mortality occurred in week 6. Prior to death, the fish were injured by rubbing against the bottom of the aquarium, causing lesions and necrosis. Examination of the fish revealed clubbed gills, hemorrhaging under the skin, fragile fins, edema, eroded barbels, rapid breathing, and swelling at the base of the pectoral fins.

As the experiment progressed, a greater number of fish demonstrated these symptoms and cumulative mortality reached 100% at the end of the experiment. After supplementation with pantothenic acid in the test diet, recovery was slow, although mortality ceased and deficiency signs disappeared after 3 weeks. Growth, however, improved only after 9 weeks on the recovery diet.

Gross postmortem examination revealed pale gills and livers. As well, the gills were covered with excessive mucous. The fish showed high concentrations of fat in the abdominal cavity and pits at the anterior part of the body just behind the skull on both sides of the occipital process.

Folic Acid Deficient Diet

The results of using this diet are illustrated in Fig. 5. The differences in weight gain and cumulative mortality between the folic acid deficient group and the complete vitamin group were significant at the end of 6 and 12 weeks respectively. Fish fed the deficient diet appeared normal, except for a slight decrease in food consumption accompanied by decreasing weight gain. Gross postmortem examination revealed fading of body colour, pale gills, and pale liver.

Niacin-Deficient Diet

The results of feeding this diet are presented in Fig. 6. The average weight gain and cumulative mortality of fish fed the niacin-deficient and complete diets were significantly different at the end of 12 and 24 weeks respectively. Loss of appetite was the first sign noted. Upon continued exposure to the deficient diet, muscle spasms, loss of equilibrium, whirling, lethargy, hemorrhaging under the skin and fins, and slightly protruding eyes were observed. Prior to death, the fish darted to the surface and immediately sank to the bottom, with some fish experiencing convulsions. After niacin was added to the diet, mortality ceased and the fish began to eat and grow normally again.

Ascorbic Acid Deficient Diet

The results of feeding this diet are illustrated in Fig. 7. There were no significant differences in weight gain or mortality between fish fed the ascorbic acid deficient diet and those on the control diet. Cumulative mortality in the deficient group was 16.66% and in the control group was 0% at the end of 24 weeks. However, the fish fed the ascorbic acid deficient diet showed scoliosis, external hemorrhaging, fin erosion, and dark skin colour at 12 weeks. Upon examination at the termination of the experiment, no abnormal features were noted in the gills, stomach, liver, kidneys, or intestines.

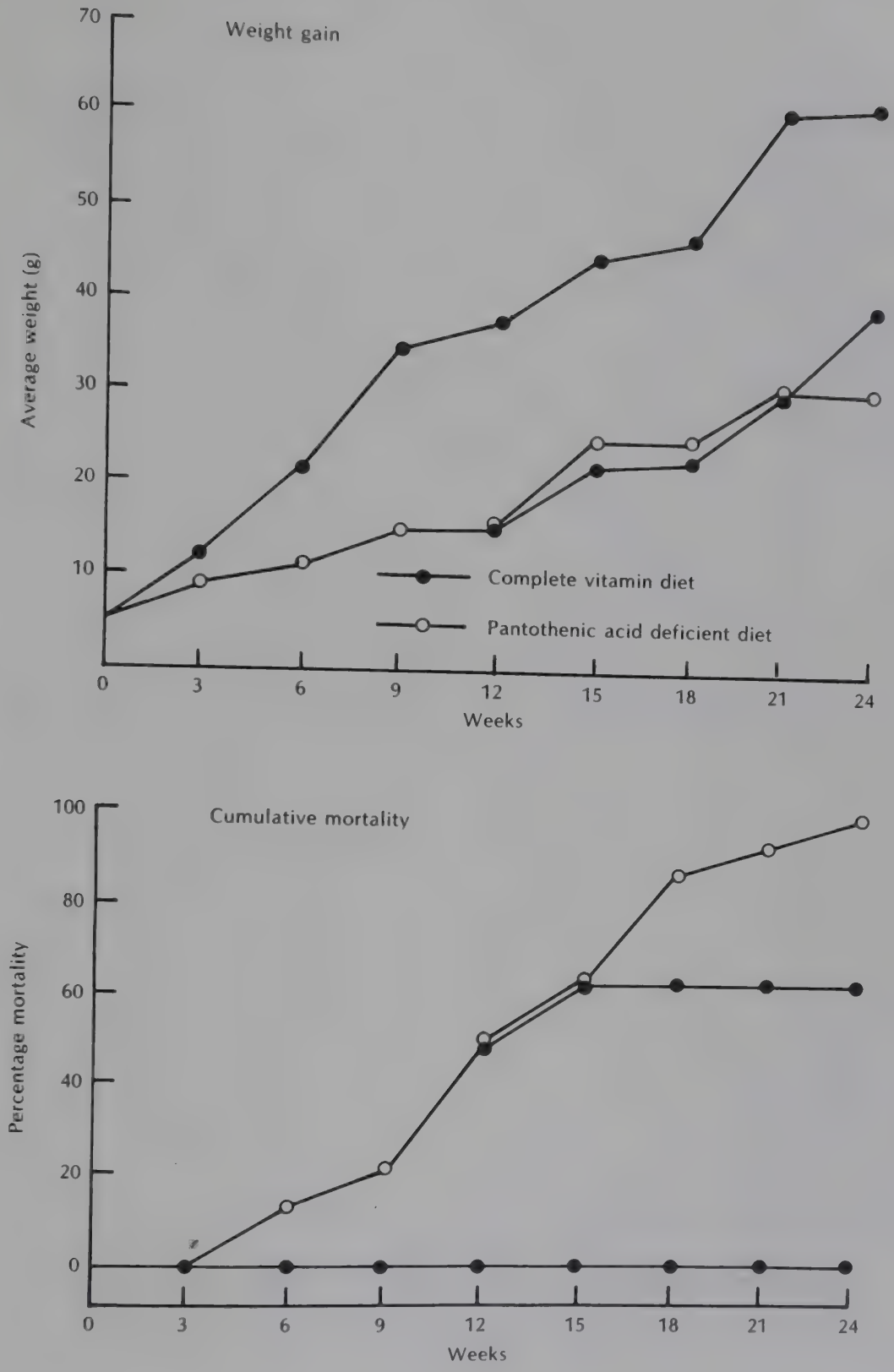
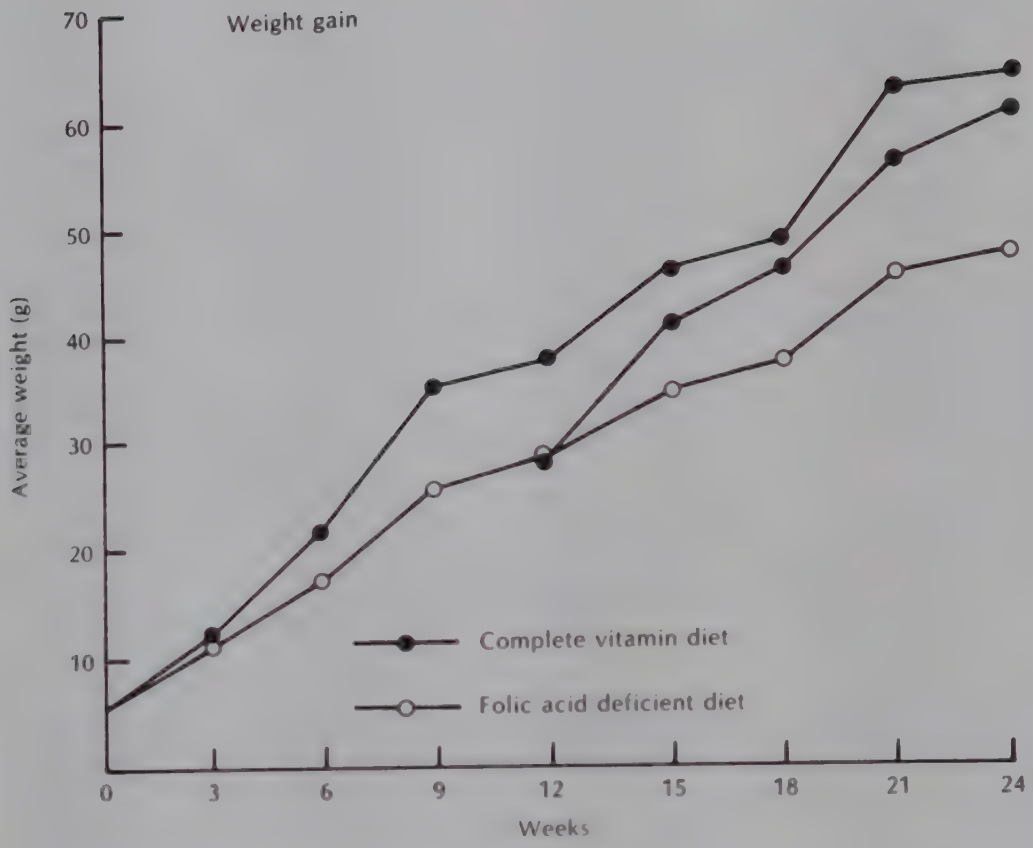


Fig. 4. Comparison of average weight gains and cumulative mortalities of Clarias *fed pantothenic acid deficient and complete vitamin diets.*



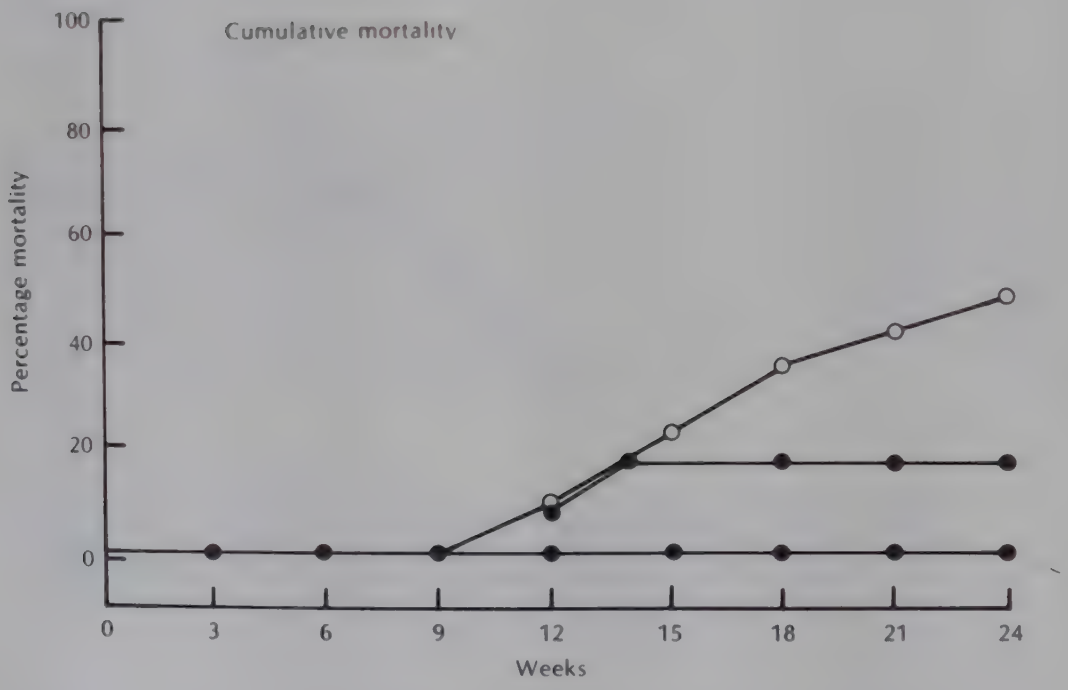


Fig. 5. Comparison of average weight gains and cumulative mortalities of Clarias *fed folic acid deficient and complete vitamin diets.*

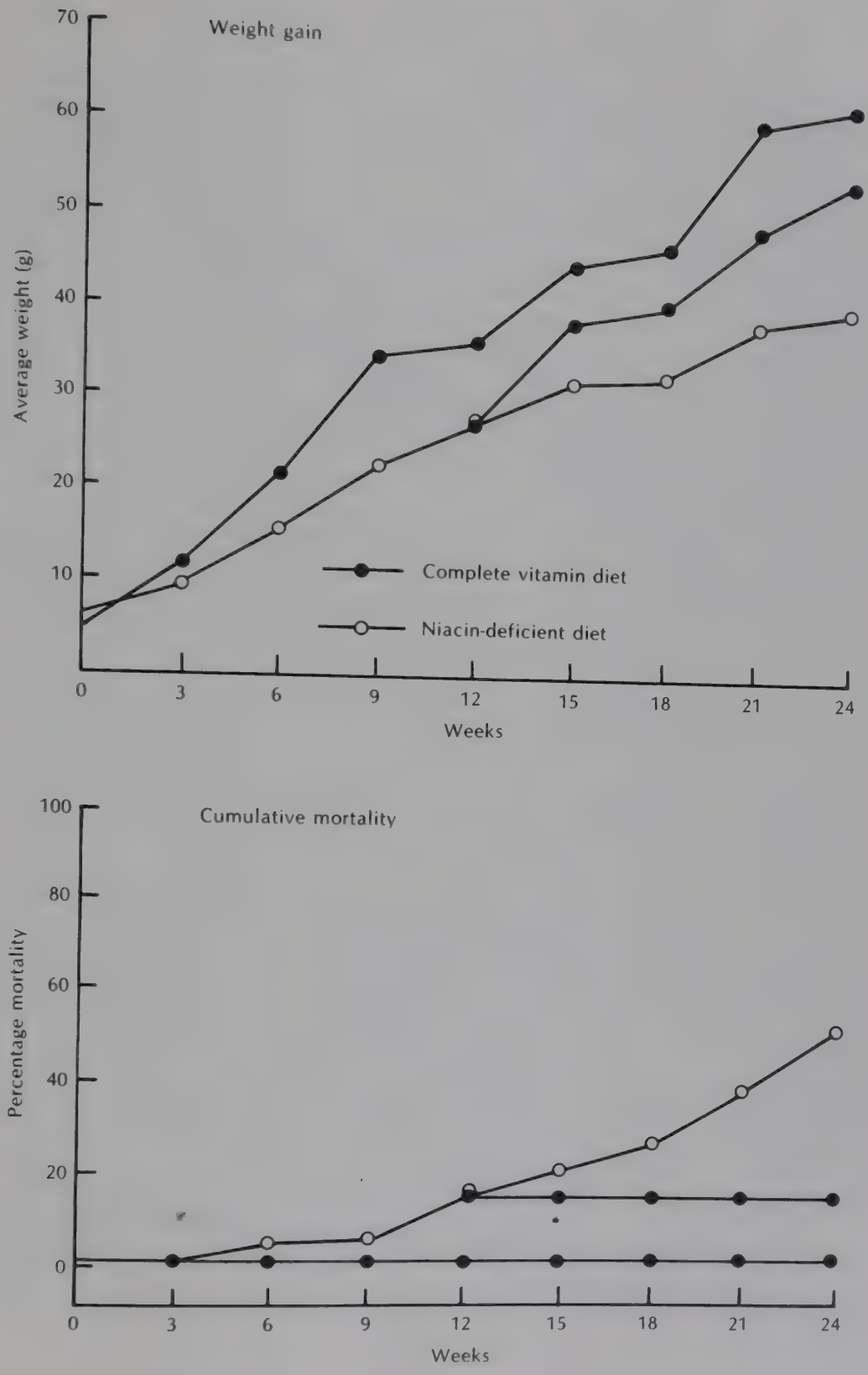


Fig. 6. Comparison of average weight gains and cumulative mortalities of Clarias *fed niacin-deficient and complete vitamin diets.*

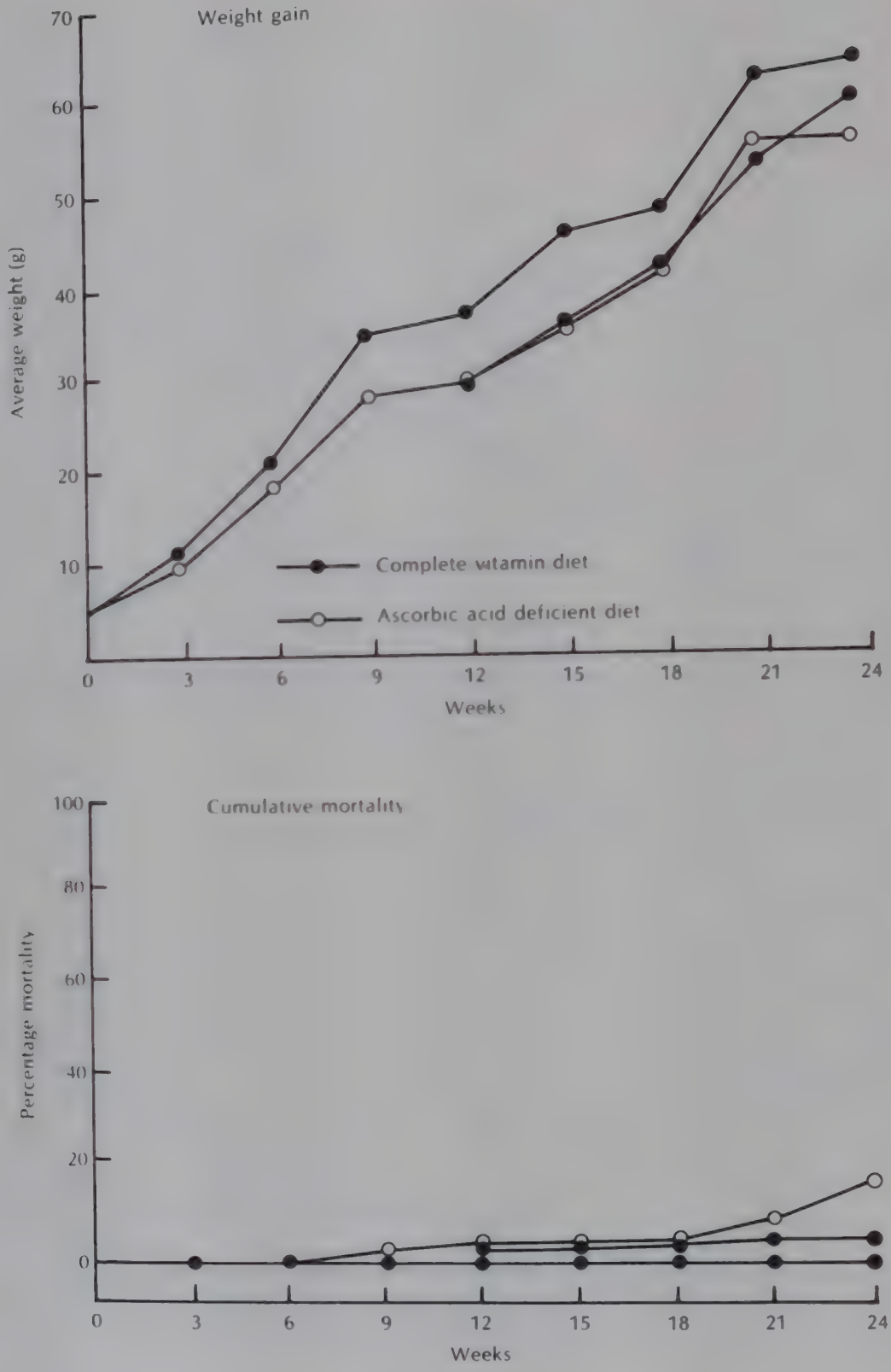


Fig. 7. Comparison of average weight gains and cumulative mortalities of Clarias *fed ascorbic acid deficient and complete vitamin diets.*

Summary

The deficiency symptoms of *Clarias* fed thiamine-deficient diets were not as severe as those observed in salmon or trout. This might be a result of the availability of B_1 produced by intestinal microorganisms. Riboflavin-deficiency symptoms in *Clarias* were similar to those in salmon, trout, and channel catfish, except the former exhibited normal growth and fading of body colour, whereas the others showed poor growth and dark skin colour. Pyridoxine was essential for both growth and survival of *Clarias*. The pantothenic acid deficient diet developed the most severe deficiency symptoms, very poor growth and eventual fatality. The swelling at the base of the pectoral fins, presumably caused by bacterial infection induced by stress due to the pantothenic acid deficiency, disappeared with time as the fish became more mature. Because folic acid is required for normal blood formation, deficiency of this vitamin caused anemia and decreased weight gain and survival in *Clarias* as well as in trout, salmon, and channel catfish. Some niacin-deficiency symptoms in *Clarias*, such as protruding eyes, were different from those observed in other species of fish. Lesions on the isthmus were one of the distinctive signs of vitamin-C deficiency in *Clarias* (Boonyaratpalin et al. 1982), but were not found in this experiment. This is probably a result of the differences in experimental conditions, age of fish, or amount of food intake.

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Determination of the Optimum Level of Vitamin Premix for the Diet of Common Carp (*Cyprinus carpio* L.) Fingerlings

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Common carp (*Cyprinus carpio* L.) fingerlings with an average individual weight of 12.4 g were stocked in fibreglass tanks filled with 150 L of water at a temperature ranging from 24.5 to 25.0°C and at a rate of 20 fish per tank. They were fed 38% protein diets containing 0, 1, 2, and 3% vitamin premix (Radjamix U) at a rate of 2.5% of the total body weight daily for a period of 8 weeks.

Fish fed the diet with no vitamin premix had significantly ($P < 0.05$) less weight gain and feed efficiency and slightly lower protein retention. Those fed diets supplemented with 1, 2, and 3% vitamin premix had similar weight gain, feed efficiency, and protein retention. The survival rates were slightly lower for fish fed 0 and 1% vitamin premix diets. The survival rates were the same for fish receiving 2 and 3% vitamin premix supplemented diets. Thus, this study shows that the addition of vitamin premix is necessary in the diet of common carp.

Introduction

Fish farming, or aquaculture, plays an important role in Third World countries as a means to alleviate malnutrition. Having realized the potential that aquaculture can offer, efforts are being made by the Indonesian government to increase fish production through expansion of culture areas and intensification of culture techniques. Thus, fish culture has changed its form from extensive to intensive methods. In the latter form of culture, the natural food supply available for fish becomes insignificant. NRC (1977) reported that supplemental vitamins in rations are important to promote normal growth of fish when they are reared intensively in ponds, raceways, or cages where natural foods are limited.

A tremendous amount of information is available regarding the nutrient requirements of common carp (*Cyprinus carpio* L.). Artificial pelletized feeds have been formulated and produced commercially in countries such as Japan, Israel, and some European countries. In Indonesia, commercial feeds are also available for use in semi-intensive culture systems but their performance is not very good and the cost is quite high. Furthermore, most farmers in Indonesia mix their own feed and micronutrients such as vitamins are sometimes omitted from the formula. Thus, this study was conducted to determine the need for supplementation of vitamin premix in

common carp fingerling diets and the effects of varying levels of vitamin premix on growth, survival, feed efficiency, and protein retention.

Materials and Methods

Facilities and Experimental Fish

Sixteen circular fibreglass tanks, located at the nutrition wet laboratory of the Research Institute for Inland Fisheries, Bogor, Indonesia, were used in this feeding experiment. Each tank had an individual air supply and was filled with 150 L of tap water (city water). Prior to use, the tap water was stored in a storage tank provided with strong aeration to eliminate chlorine. Each fibreglass tank was equipped with a standpipe drain for controlling the water level. About two-thirds of the water was changed twice daily (in the morning and afternoon). The water temperature ranged from 24.5 to 25°C. Dissolved oxygen concentration varied from 5.6 to 7.9 ppm.

Common carp fingerlings with an average weight of 12.4 g were used in the experiment. They were acclimatized for 1 week prior to the beginning of the experiment. During this period, they were fed a supplemental diet containing 38% crude protein. They were randomly selected and stocked at a rate of 20 fish per tank.

Experimental Diets

Four diets were formulated to contain 38% crude protein and 7.5% crude fat (Tables 1 and 2). They contain other nutrients at the same level; only the amount of the vitamin premix differed. Poultry vitamin premix (Radjamix U)[1] was used. This was added to each of the four diets at levels of 0, 1, 2, and 3%.

The dry ingredients of each diet were mixed in a Hobart mixer for a sufficient period of time. The oil was gradually added to the dry mixture and the whole batch was mixed thoroughly for another 5 min to assure homogeneity. A sufficient amount of water was added and the resulting moist mixture was extruded through a 2-mm diameter die in a meat grinder. The moist pellets were sun-dried and stored in separate plastic containers.

Management

This experiment consisted of four treatments with four replications per treatment. Each diet was given to the 20 fish contained in each of the four tanks. The fish were fed three times daily, one-third of the ration in the morning at 0800 hours, one-third at noon (1200 hours), and another one-third in the afternoon at 1600 hours. The experimental fish were fed 7 days a week at a rate of 2.5% of their body weight per day. The fish in all tanks were weighed and counted every 2 weeks to estimate weight gain and survival rate. Initially, calculations of total daily feed allowances were based on the average weight of the fish in all treatments. Thereafter, the feed allowances were adjusted every 2 weeks based on the average weight of the fish in each treatment and the numbers of fish in each tank. The fish were given the experimental diets for a period of 8 weeks. About two-thirds of the water in each tank was changed every morning and afternoon by siphoning

[1] The use of this trade name does not imply endorsement of the product.

Table 1. Percentage composition of the four experimental diets containing different levels of vitamin premix.

Ingredient	Diet			
	1	2	3	4
Fish meal	58.2	58.5	58.9	59.2
Wheat flour	10.0	10.0	10.0	10.0
Rice bran	13.0	11.7	10.3	9.0
Corn meal	10.0	10.0	10.0	10.0
Blood meal	5.0	5.0	5.0	5.0
Fish oil	2.8	2.8	2.8	2.8
Mineral premix	1.0	1.0	1.0	1.0
Vitamin premix[a]	0.0	1.0	2.0	3.0
Calculated crude protein (%)	38.0	38.0	38.0	38.0
Calculated crude fat (%)	7.5	7.5	7.5	7.5

[a] Poultry vitamin premix Radjamix U was used and the levels of vitamins in the different experimental diets are shown in Table 2.

to remove all of the wastes. At biweekly intervals, when the fish were removed for weighing, the tanks were drained and cleaned thoroughly.

During the course of the experiment, the drainpipe in the tank with the 3% vitamin premix treatment slipped away. The water drained completely and mass mortality of the fish occurred.

Statistical Design and Analyses

The experiment consisted of four treatments with four replications each. The treatments were assigned randomly using a completely randomized design. Determination of the optimum dietary level of vitamin premix was based on weight gain, survival, feed efficiency, and protein retention. Statistical analyses of the data were carried out using the single classification anova with missing data (Steel and Torrie 1980).

When a significant difference was found among the various treatments, Duncan's multiple-range test was used to detect the differences between treatment means.

Table 2. Composition of supplemental vitamins in 1-kg experimental diets.

Vitamin	Diet[a]			NRC recommendation[b] (complete)
	2	3	4	
A (IU)	20000	40000	60000	5500
D_3 (IU)	2000	4000	6000	1000
E (IU)	5	10	15	50
K (mg)	2	4	6	10
Choline chloride (mg)	6	12	18	550
Nicotinamide (mg)	40	80	120	100
Riboflavin (mg)	12	24	36	20
Pyridoxine (mg)	0.5	1	1.5	20
Thiamine (mg)	2	4	6	20
D-calcium pantothenate (mg)	6	12	18	50
Folacin (mg)	0.2	0.4	0.6	5
Ascorbic acid (mg)	0	0	0	30–100
Inositol (mg)	0	0	0	100
B_{12} (μg)	12	24	36	20
Biotin (μg)	10	20	30	100

[a] Diet 1 was not supplemented with vitamin premix.
[b] Source: NRC (1977).

Results and Discussion

The growth curves of common carp fingerlings fed diets containing different levels of vitamin premix for an 8-week period are presented in Fig. 1. During the first 4 weeks of the study, the fish in all treatments grew at approximately the same rate. After the 6th and 8th weeks, the growth of the fish in the treatment with no supplemental vitamin premix decreased significantly. Fish receiving 1, 2, and 3% vitamin premix grew at about the same rate throughout the experimental period.

The average weight gain, survival rate, feed efficiency, and protein retention of the fish in the various treatments are given in Table 3. Fish fed the diet with no vitamin premix had a weight gain of 9.78 g, which was significantly lower ($P < 0.05$) than that of the other treatments. No significant differences were found among the weight gains of fish fed with diets containing vitamin premix. These results indicate that the inclusion of the vitamin premix in the diet is beneficial for the growth of common carp fingerlings. Stickney (1979) indicated that vitamins are essential for the growth of fish, although the quantity required is very small. Anggorodi (1979) reported that vitamins are very important for promoting growth and building new body cells.

Table 3. Averages of weight gain, survival rate, feed efficiency ratio, and protein retention of common carp fingerlings fed diets containing various levels of vitamin premix.

Treatment (level of vitamin premix)	Replication	Average weight gain (g)	Survival rate (%)	Feed efficiency (%)	Protein retention (%)
0	1	11.4	65	2.42	—
	2	7.7	100	2.95	7.77
	3	8.9	100	2.59	10.68
	4	11.1	100	2.13	19.47
		(9.78[a])	(91.25)	(2.49[a])	(12.64)
1	1	16.2	100	1.45	23.66
	2	15.8	95	1.55	20.52
	3	14.4	100	1.61	17.44
	4	13.9	60	1.54	—
		(15.08[b])	(88.75)	(1.53[b])	(20.54)
2	1	16.1	100	1.47	20.58
	2	15.1	100	1.54	—
	3	14.6	100	1.59	19.30
	4	16.0	100	1.48	25.80
		(15.45[b])	(100.00)	(1.52[b])	(21.89)
3	1	15.5	100	1.49	23.73
	2	15.8	100	1.49	20.46
	3	14.6	100	1.58	19.66
		(15.30[b])	(100.00)	(1.52[b])	(21.89)

Note: Average values in parentheses. Means followed by the same superscript are not significantly different at $P > 0.05$.

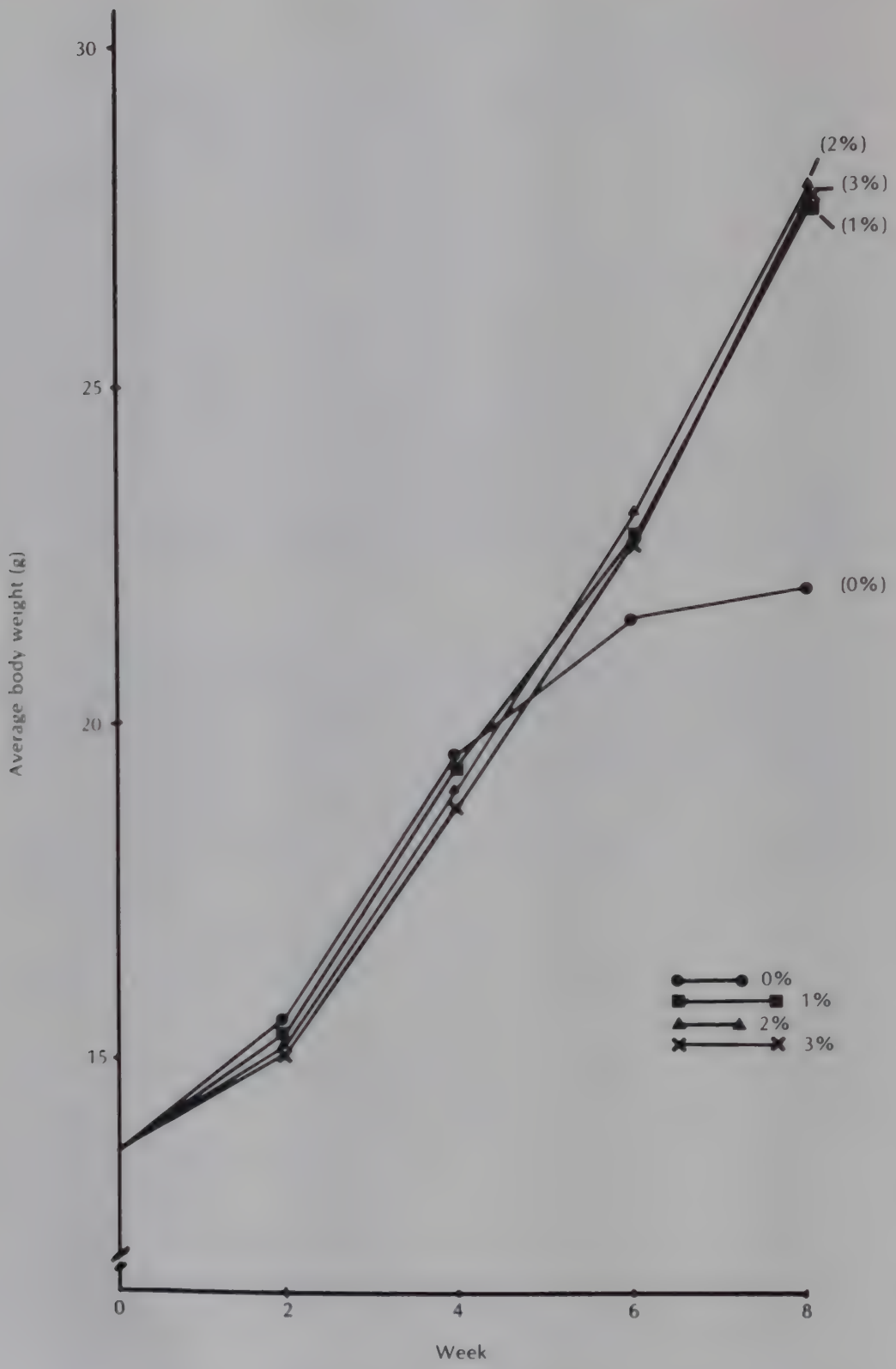


Fig. 1. Growth curve of common carp fingerlings fed diets containing various levels of vitamin premix.

The survival rates of the fish were 91.25, 88.75, 100, and 100% for the treatments receiving diets supplemented with 0, 1, 2, and 3% vitamin premix respectively. No significant differences ($P > 0.05$) were found among these values even though there were some incidences of mortality among those fish receiving 0 and 1% vitamin premix.

The feed efficiency was significantly lower for the treatment with no vitamin supplement (2.49%). Feed efficiency values were similar for the other three diets. This indicates that the addition of vitamin premix to the diet significantly improved the nutritive value of the diet.

The protein retention of fish fed the vitamin premix deficient diet was the lowest at 12.64%. The retention values were the same for fish fed the diets containing 2 and 3% vitamin premix. The fish fed the 1% vitamin premix diet had an intermediate value of protein retention (20.54%). These values were not significantly different.

Although no significant differences could be detected among the protein retention values of fish fed the diets containing various levels of vitamin premix, the lower values obtained in those treatments with 0 and 1% vitamin supplement may indicate that insufficient quantities of vitamins were received for proper protein metabolism.

The results obtained from this experiment indicate that supplementation of vitamin premix in the diets is necessary for good growth and feed efficiency. Protein retention in fish is likewise improved by diets containing vitamin premix. Although supplementation of poultry premixes improved the growth of the common carp in this study, the premix for the fish should be based on NRC (1977) recommendations. Furthermore, many of the poultry vitamin premixes contain trace minerals that, depending on the level in the premix, could be very toxic to the fish.

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Acceptability of Five Species of Freshwater Algae to Tilapia (*Oreochromis niloticus*) Fry

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Unialgal cultures of *Oscillatoria quadripunctulata*, *Chroococcus dispersus*, *Navicula notha*, *Euglena elongata*, and *Chlorella ellipsoidea* were fed to tilapia fry for 30 days. Mean weights and survival rates of the fry were highest when given *Navicula* (105.6 mg, 86%) and *Chroococcus* (89.1 mg, 90%). *Oscillatoria*, a filamentous cyanophyte, showed limited acceptability to tilapia fry, possibly because of its larger size in comparison with *Chroococcus*. Fry fed *Chlorella* and *Euglena* did not survive at all.

C^{14}-labeled algae of the above species were fed to tilapia fry of varying ages. Assimilation rates per fry after 24 hours of feeding with a suitable algal species increased with the age of the fry. Moreover, the same trend as in the growth and survival experiments was observed, i.e., the highest assimilation rates were obtained in 40-day old tilapia fry given *Navicula* and *Chroococcus* as natural feeds. On the other hand, negligible amounts of the other three algal species tested were assimilated by tilapia fry.

The above results were explained in terms of the enzyme secretion of tilapias. There seemed to be no transition stage in the feeding habit of both fry and adult tilapia. The acceptability of plant matter in the diet of even the early larval stages was demonstrated.

Introduction

Increasing demand for tilapia fingerlings is based on their wide acceptance as a major source of animal protein in many Asian countries. In the Philippines alone, millions of fingerlings are needed for stocking in pens and cages. To meet this demand, the existing backyard hatcheries in the country will have to expand and improve their larval-rearing techniques. Providing fry with the most suitable algal food would enhance growth and survival, thereby reducing production costs.

At a workshop on induced fish breeding in Southeast Asia (IDRC 1981), the natural food requirement was identified as one of the problem areas in larval rearing. Reports from different countries showed that animal protein, mostly *Moina* and *Daphnia*, has commonly been used as larval food for freshwater species. On the other hand, about 40 algal species have been tested for nursery rearing of juvenile bivalves in other countries (De Pauw 1981). The food value of algae differed even among related species within

the same class, family, or genus.

This paper describes which among five species representing four algal divisions is most acceptable to tilapia fry based on their growth and survival rates as well as assimilation rates of C^{14}-labeled algae.

Materials and Methods

Growth and Survival Experiment

Newly hatched tilapia (*O. niloticus*) fry, with yolk already absorbed and having initial measurements of 10 mm and 0.7 mg, were stocked in gallon jars containing 2 L of tap water and 5 fry/L. They were supplied with unialgal cultures of: (1) *Chroococcus dispersus*, (2) *Oscillatoria quadripunctulata*, (3) *Navicula notha*, (4) *Chlorella ellipsoidea*, and (5) *Euglena elongata*. Algal cell densities of the above species were maintained at 20 000–60 000 cells/mL.

Rearing containers were cleaned daily and the water was changed and replenished with a new supply of algae each day. Water quality, in terms of NH_3-N and pH, was analyzed weekly using APHA standard methods and a pH meter. The temperature was taken with an ordinary thermometer. Algal cell density was determined using a haemacytometer.

The length and weight of the fry were measured at 10-day intervals using the weight displacement method with a Mettler analytical balance. Daily mortalities and the final survival rates were also recorded. The experiment was conducted from 25 August – 29 September 1982.

Labeling of Algae with C^{14}

Unialgal cultures of selected species were maintained in a sterile BRSP-M_1 medium. For C^{14} labeling, an ampoule containing 1 mL of 20.24 μCi $NaHC^{14}O_3$ with 44 939 000 dpm/mL[1] was emptied into 170 mL of algal culture at the logarithmic phase. Cultures and radioisotopes were shaken vigorously and incubated for 6 hours with fluorescent light at a light intensity of 2760 lux.

Before feeding, 10 mL of C^{14}-labeled algae was filtered through a Millipore filter (0.45 μ) and washed three times with distilled water. The algae were then oven-dried for 6 hours at 100°C. The dry weight of algae per treatment at different growth stages and their corresponding activity were determined.

The remaining labeled algae were centrifuged, washed three times in sterile distilled water, and fed to fry.

Feeding of Tilapia Fry

Tilapia fry from the growth and survival experiment were taken at 0, 10, 20, 30, and 40 days and designated as stage 0, I, II, III, and IV respectively. The fry were starved for 24 hours prior to feeding. High-density cultures of C^{14}-labeled algae were supplied for 24 hours to the growing tilapia fry. The fry were thoroughly washed in running water, transferred to another container, and allowed to feed on unlabeled algae for another 24 hours prior to sacrificial sampling.

[1] Obtained from the Carbon[14] Center, Horsholm, Denmark.

Preparation of Samples for Liquid Scintillation Counting

Samples were exposed to formalin fumes for 1 min for fixation and 6 *N* HCl fumes for 3 min to remove unassimilated $Na_2HC^{14}O_3$ through the release of $C^{14}O_2$. Samples were digested in 1 mL of 6 *N* NaOH for 6 hours at 100°C in a water bath. LSC cocktail consisting of PPO (2,5-diphenyloxazole), 4 g; POPOP (1,4-bis-2-(5-phenyl oxazolyl)-benzene), 200 mg; toluene, 500 mL; and Triton X, 500 mL, was added at 7 mL per vial. The same amount of NaOH and scintillant were added in the preparation of the quench correction curve, which was used in determining the counting efficiency. Sample activity was read with a Beckman LS-150 liquid scintillation counter.

Results

Growth and Survival of Tilapia Fry Given Different Algae

The acceptability and preference of tilapia fry for the unicellular species *Chroococcus dispersus* and *Navicula notha* were evident throughout the larval-rearing period (Fig. 1). As early as during the first 10 days of growth, the mean weights of tilapia larvae given these algae as natural feeds were 22.8 and 23.3 mg for *Chroococcus* and *Navicula* respectively (Table 1). In contrast, tilapia fry fed *Oscillatoria* attained only about half of the above mean weight (13.4 mg), whereas those given *Chlorella* and *Euglena* attained even lower weights (7.2 and 7.3 mg). Growth continued to accelerate with time in the two treatments using *Navicula* and *Chroococcus*, although there was a slight decrease in weight on the 35th day for the treatment using *Chroococcus*. *Oscillatoria* seemed to have very limited acceptability. Continuous feeding with *Oscillatoria* resulted in very slow growth. Fry fed *Oscillatoria* reached only 18% of the weight gain attained in the treatment using *Navicula*. Finally, fry fed *Chlorella* and *Euglena* did not survive

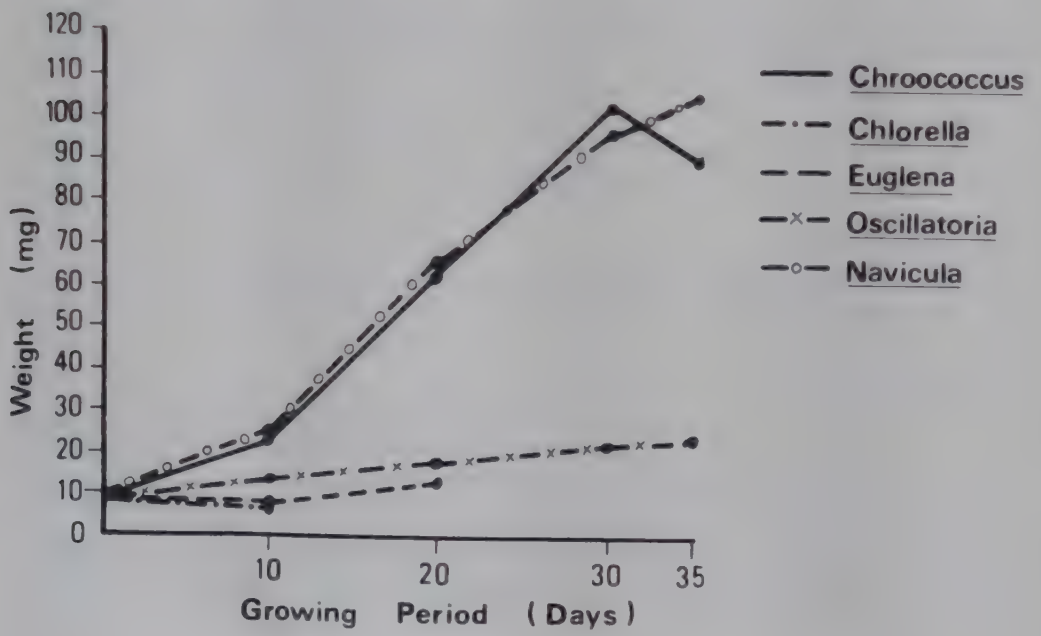


Fig. 1. Growth of tilapia (O. niloticus) *fry given five species of freshwater algae.*

Table 1. Duncan's new multiple-range test for mean weight (mg) ($\alpha = 0.05$) of *O. niloticus* fry at different growth stages fed with different algae.

Algal species	Stage I (10 days old)	Stage II (20 days old)	Stage III (30 days old)	Stage IV (35 days old)
Chlorella	7.23^{a}	0^{a}	0^{a}	0^{a}
Euglena	7.3^{a}	2.12^{b}	0^{a}	0^{a}
Oscillatoria	13.41^{b}	8.58^{c}	22.85^{b}	24.67^{b}
Chroococcus	22.82^{c}	63.92^{d}	100.47^{d}	94.97^{c}
Navicula	23.27^{c}	67.13^{e}	97.25^{c}	131.38^{d}

Note: Means followed by the same superscript are not significantly different.

beyond 10 and 20 days respectively.

Statistical analyses revealed significantly high weight increments in tilapia fry fed *Navicula* in almost all growth stages (Table 1). The treatment using *Chroococcus* ranked second to *Navicula*, except for stage III fry, which showed the highest weight gain. Significantly moderate weight increases were obtained in the treatment using *Oscillatoria*. The lowest weight increments were obtained in the treatments using *Chlorella* and *Euglena*.

Survival rates of *O. niloticus* fry followed the same trend as growth, i.e., the highest survival rate was obtained with *Navicula* and *Chroococcus* (Fig. 2). It should be noted that very young tilapia fry (up to 10 days old) seem to survive well in a medium containing *Oscillatoria*. There was increasing mortality of fry, however, with continuous feeding of *Oscillatoria*, so that only about half (48%) of the population remained by the fingerling stage. With the other algae being used as feed, consistently low survival rates were obtained.

Significantly high survival rates were obtained in all growth stages of tilapia fry fed *Navicula* and *Chroococcus* (Table 2). The treatment using *Oscillatoria* gave equally high survival rates for young tilapia fry; at stage IV, however, high mortality occurred.

Crude protein analysis of the different algae used in this experiment

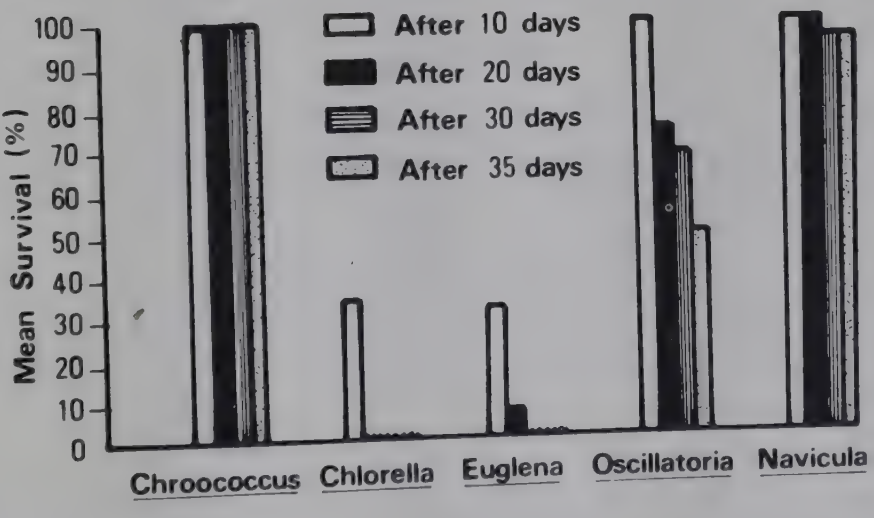


Fig. 2. Mean percentage survival of O. niloticus *fry given five species of algae.*

Table 2. Duncan's new multiple-range test ($\alpha = 0.05$) for mean percentage survival of *O. niloticus* fry fed with five algal species at different growth stages.

Algal species	Stage I (10 days old)	Stage II (20 days old)	Stage III (30 days old)	Stage IV (35 days old)
Chlorella	33.3[a]	0[a]	0[a]	0[a]
Euglena	36.7[a]	3.3[a]	0[a]	4.3[b]
Oscillatoria	100.0[b]	73.3[b]	63.3[b]	90.0[c]
Chroococcus	100.0[b]	100.0[c]	96.7[b]	86.7[c]
Navicula	96.7[b]	96.7[c]	93.3[b]	86.7[c]

Note: Means followed by the same superscript are not significantly different.

showed that *Euglena* and *Chroococcus* contained the highest protein, 35.68 and 30.38% respectively (Table 3). Grown under laboratory conditions in an inorganic medium, *Chlorella* had a relatively low crude protein content (19.45%).

Two important chemical parameters were monitored in the rearing medium containing different algae (Table 4). The pH did not vary much among the different treatments. However, there were differences in the unionized ammonia that was calculated at 28°C, the temperature of the water throughout the experiment. Relatively high concentrations were observed in the rearing medium containing *Chlorella* at stage I and *Chroococcus* and *Oscillatoria* at stage III. However, the values did not reach levels considered to be toxic to the fish.

Assimilation Rates of Tilapia Given Different Algal Feeds

Feeding of C^{14}-labeled algae enabled assimilation rates of tilapia fry to be quantified (Table 5). Results showed that increasing amounts of *Chroococcus* and *Navicula* were assimilated as *O. niloticus* fry grew to the fingerling stage (Fig. 3).

Based on the computed amount of algae assimilated per day, 40-day old fry assimilated as much as 4.93 mg *Chroococcus* and 4.2 mg *Navicula* in 24 hours on a dry-weight basis (Table 5). The three other species consistently gave very low assimilation rates; although the assimilation of *Oscillatoria* was higher than that of *Chlorella* or *Euglena*. The difference in the assimilation rates of the different algae is highly significant (Table 6).

Discussion

The results established the possibility of feeding the diatom *Navicula notha* and blue–green alga *Chroococcus dispersus* to tilapia fry to produce fingerlings. The acceptability of these two species may be explained in

Table 3. Protein content of algae.[a]

Algae	Crude protein (% dry weight)
Chroococcus	30.38
Oscillatoria	25.6
Navicula	26.77
Euglena	35.68
Chlorella	19.45

[a] Data courtesy of the Analytical Service Laboratory, Department of Animal Science, University of the Philippines at Los Baños.

Table 4. Range of total NH_3-N, unionized NH_3, and pH in milkfish-rearing medium supplied with different algae.

Growth stage	Chemical parameters	Algal food				
		Chroococcus	*Chlorella*	*Euglena*	*Oscillatoria*	*Navicula*
I	Total NH_3-N(ppb)	15.4–118	382–457	13–12.2	7.4–10.6	50–133
	Unionized NH_3(ppb)	6.63–58.6	100–169	5.6–6.06	3.18–5.26	15.6–49.1
	pH	8.9–9.0	8.6–8.8	8.9–9.0	8.9–9.0	8.7–8.8
II	Total NH_3-N(ppb)	75.9–210	8.6–41.6	34–201	4.3–6.9	10.0–328
	Unionized NH_3(ppb)	13.6–55.1	1.54–10.9	6.11–86.6	0.8–2.6	1.8–163
	pH	8.4–8.6	8.4–8.6	8.4–8.9	8.4–8.8	8.4–9.0
III	Total NH_3-N(ppb)	534–1740	—[a]	—	563–680	115–690
	Unionized NH_3(ppb)	140–642	—	—	434–606	35.9–255
	pH	8.6–8.8	—	—	9.4–9.6	8.7–8.8
IV	Total NH_3-N(ppb)	561–693	—	—	7.8–6.3	34–123
	Unionized NH_3(ppb)	122–182	—	—	4.0–4.41	10.63–45.4
	pH	8.5–8.6	—	—	9.1–9.2	8.7–8.8

[a] All fish died.

Table 5. Computation of assimilation rates of different C^{14}-labeled algal food by *O. niloticus* fry at various growth stages.[a]

Growth stage		Treatment				
		Chroococcus	*Chlorella*	*Oscillatoria*	*Euglena*	*Navicula*
0	Corrected cpm/mg algae[b]	9522.23	50088.39	125363.57	85718.3	19881.91
	Corrected fish cpm/24 hours[b]	11141.2	1463.28	5496.72	251.50	9559.79
	Algal assimilation (mg, dry weight/24 hours)[c]	0.674	0.011	0.054	0.034	0.565
	Mean fish weight (mg)	0.7	0.7	0.7	0.7	0.7
I	Corrected cpm/mg algae[b]	4085.7	5704.5	4957.10	10401.1	3881.72
	Corrected fish cpm/24 hours[b]	8258.35	188.21	801.2	1070.09	6573.88
	Algal assimilation (mg, dry weight/24 hours)[c]	1.011	0.016	0.081	0.051	0.847
	Mean fish weight (mg)	7.2	7.3	6.8	6.4	7.3
II	Corrected cpm/mg algae[b]	4119.75	5752.07	4998.4	8390.22	3131.26
	Corrected fish cpm/24 hours[b]	11224.95	287.60	1214.60	1255.73	8934.53
	Algal assimilation (mg, dry weight/24 hours)[c]	1.360	0.025	0.122	0.075	1.426
	Mean fish weight (mg)	8.6	8.9	8.8	8.3	8.7
III	Corrected cpm/mg algae[b]	4064.82	5675.38	4931.77	8278.97	3088.96
	Corrected fish cpm/24 hours[b]	9972.36	264.00	1077.92	1114.0	7920.09
	Algal assimilation (mg, dry weight/24 hours)[c]	2.453	0.045	0.219	0.135	2.564
	Mean fish weight (mg)	22.9	23.8	22.6	23.7	22.9
IV	Corrected cpm/mg algae[b]	4064.86	5674.85	4931.42	9520.82	3088.96
	Corrected fish cpm/24 hours[b]	69460.33	302.66	1183.54	1361.46	40273.0
	Algal assimilation (mg, dry weight/24 hours)[c]	4.938	0.053	0.240	0.143	4.230
	Mean fish weight (mg)	38.6	37.5	39.3	36.5	39.4

[a] Data are means of three replicates.

[b] Corrected counts = LSC counts/counting efficiency.

[c] Algal assimilation (mg, dry weight) 24 hours = $\frac{\text{corrected fish cpm/24 hours}}{\text{corrected algal feed cpm/mg}}$

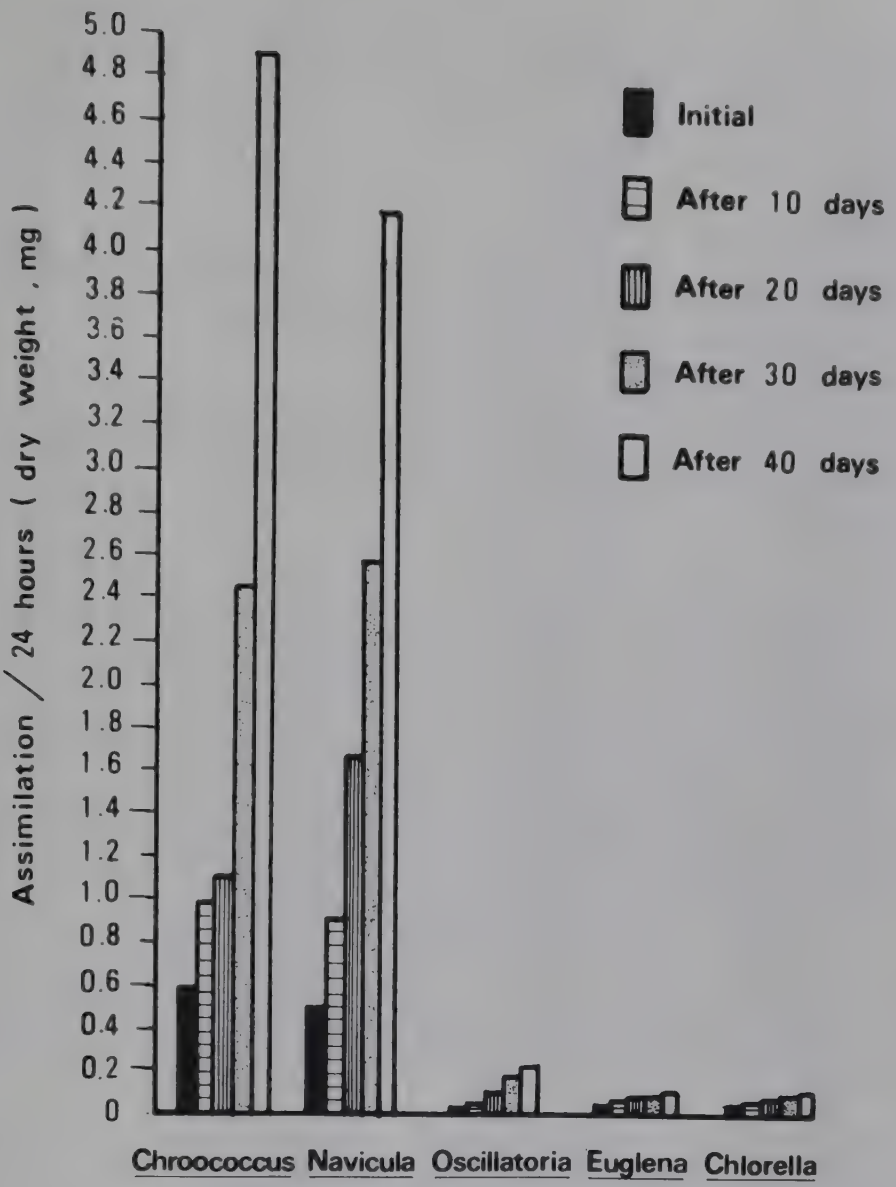


Fig. 3. Assimilation rates of tilapia (O. niloticus) *fry at various growth stages given* C^{14}*-labeled algae.*

terms of the gastric-acid secretion of tilapias. Earlier reports indicated that tilapias secrete gastric acid at very low pH, which increases digestion of diatoms (Bowen 1976). Furthermore, exposure to acid at a pH of less than 2 also decomposes chlorophyll as well as blue–green algal cell walls (Moriarty 1973).

Much of the earlier work on the feeding habit of this fish dealt with the diet of adult tilapias in their natural habitat (Moriarty and Moriarty 1973; Caulton 1977). It was assumed that, similar to other fish, the larvae, fry, and early juvenile stages of tilapia feed on small invertebrates (Le Roux 1956). However, the larval rearing experiments conducted in this study showed the acceptability of phytoplankton, specifically the unicellular species *Navicula* and *Chroococcus*. The acceptability of plant protein, even at the very early larval stages of tilapia, seemed to suggest that there is no transition stage in the feeding habit. The diet of both larvae and adult tilapia

Table 6. Duncan's new multiple-range test ($\alpha = 0.05$) for square root ($\sqrt{x}$) transformed data of algal assimilation by *O. niloticus* at different growth stages.

Algal species	Stage I (10 days old)	Stage II (20 days old)	Stage III (30 days old)	Stage IV (40 days old)
Euglena	0.036[a]	0.223[a]	0.274[a]	0.367[a]
Chlorella	0.103[a]	0.126[a]	0.155[a]	0.208[a]
Oscillatoria	0.122[b]	0.283[a]	0.347[a]	0.465[a]
Chroococcus	0.623[c]	0.946[b]	0.158[b]	1.554[b]
Navicula	0.690[c]	0.920[b]	1.187[b]	1.590[b]

Note: Means followed by the same superscript are not significantly different.

are similar, consisting primarily of plant matter.

Adult tilapias are reported to subsist on green algae, blue–green algae, diatoms, and macrophytes (Bowen 1982). Unlike the adult, our findings show that tilapia larvae/fry are selective. Of the five species tested as natural feeds, only *Navicula* and *Chroococcus* resulted in enhanced growth and survival of fry. Moreover, *Oscillatoria*, which is also a cyanophyte like *Chroococcus* but filamentous, was of very limited acceptability to tilapia fry. This may be explained by the relatively larger size of the filaments of *Oscillatoria* compared with those of *Chroococcus*. In the case of the other two species tested, the poor results may be due to factors such as toxicity and cell-wall composition of the species.

Results of feeding C^{14}-labeled algae to tilapia fry were consistent with the growth and survival experiments. The highest assimilation rates were obtained by feeding *Navicula* and *Chroococcus* during all growth stages of the tilapia larvae/fry. These results are supported by earlier reports that the assimilation efficiency values of tilapia feeding on natural diets of blue–green algae and diatoms are high (Bowen 1982).

Acceptability of selected freshwater algae for larval rearing of other economically important fish species (e.g., milkfish, carp) is the subject of ongoing experimentation in our laboratory. Basic research is also being pursued to determine the subcellular changes in algal cells consumed by fish larvae up to the fingerling stage.

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Lipid Composition of Milkfish Grown in Ponds by Traditional Aquaculture

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Milkfish is the most important cultured food fish in the Philippines, Taiwan, and Indonesia. Traditional milkfish aquaculture in the Philippines depends upon two natural food bases: one consists of a complex of unicellular algae and diatoms; the other consists of filamentous green algae, predominantly *Chaetomorpha brachygona*. The lipid composition of milkfish reared on a unicellular algal complex (sample A) and on filamentous algae (sample B) was determined. The fatty-acid pattern of the depot fat of both samples of milkfish reflected the diet. However, the fatty-acid composition of lipids from milkfish liver showed a marked increase in the levels of long-chain polyunsaturated fatty acids of the $\omega 3$ and $\omega 6$ series. This suggests that there was a metabolic transformation of dietary fatty acids in the liver. Although milkfish grown by traditional aquaculture feed on natural food that has relatively low lipid content, the fish actively metabolize and accumulate lipids through active metabolic transformation not only of available dietary lipids but also of the nonlipid nutrient components, such as carbohydrates and proteins.

Introduction

Milkfish is one of the most important food fish in Southeast Asia. It is widely cultured in Indonesia, the Philippines, and Taiwan (Chen 1976). In the Philippines, about 90% of the total aquaculture production comes from milkfish culture (BFAR 1976).

Traditional aquaculture techniques are still favoured by many Philippine fish farmers. These techniques rely on cultivation of natural food bases. As suitable areas for aquaculture become a limiting factor, an increase in productivity could be brought about by the use of artificial diets. Fundamental studies on nutrient requirements and metabolism are desirable to formulate artificial diets for aquaculture. Most fish are known to efficiently digest and metabolize lipids. However, there is no information on lipid metabolism, composition, and requirement of milkfish. This study, therefore, compares the lipid composition of milkfish grown on two different natural food bases by traditional aquaculture.

Materials and Methods

Fish Samples

Milkfish samples weighing about 200–300 g and grown on two types of natural food bases were collected from private fish ponds in Panay Island, Philippines. Each batch consisted of about 60 randomly sampled fish. After capture, the fish, packed in ice, were transported to the laboratory. The fish were dissected within 48 hours after capture.

Extraction of Total Lipids

The total lipids of individual livers and depot fat in the muscle of the belly section from each batch of fish and samples of natural food from the ponds where the fish were grown were extracted and purified using the method of Bligh and Dyer (1959). The iodine value of the total lipids was determined by the Wijs method as modified by Griffins (1968).

Lipid classes were resolved by column chromatography using silicic acid (Moerck and Ball 1973). Neutral lipids were fractionated further by column chromatography using florisil (Carroll 1961).

The total lipids were saponified and transesterified with 0.5 *N* methanolic sodium hydroxide and boron trifluoride-methanol reagent (Metcalfe et al. 1966). The fatty acid methyl esters were analyzed by gas–liquid chromatography on a Shimadzu GC-4C gas chromatograph using a flame ionization detector. The column was packed with 15% diethyleneglycol succinate (DEGS) on a 100-mesh acid-washed Chromosorb ω support. The column was made of stainless steel (3 m long, 3 mm i.d.) and the column temperature was set at 180°C. Chromatographic standards of polyunsaturated fatty acid methyl esters (No. 4-7033 and No. 4-7015) were purchased from Supelco, USA.

Results

Table 1 shows the physicochemical condition of the pond waters and the mean weight of the fish at the time of sampling. The fish samples were grown on two different types of natural food. Fish in sample A were grown on a community of unicellular algae and diatoms, whereas fish in sample B were fed fibrous filamentous green algae composed predominantly of *Chaetomorpha brachygona*. Both ponds had comparable values for parameters such as water temperature, pH, and dissolved oxygen but differed markedly in salinity. The salinity was higher in the pond from which fish in sample A were collected. The typical nutrient composition of the natural food bases is shown in Table 2.

Total Lipid Content and Iodine Number

Table 3 shows the total lipid content of the natural food, milkfish liver, and depot fat. The natural food contains very little lipid. The milkfish carcass has a lipid content of about 4.9% on a wet-weight basis. The depot fat generally contained more lipid than the liver. However, the total lipid content and iodine number for specific milkfish tissues were comparable. The iodine number of milkfish liver was higher than the lipids from the natural food.

Table 1. Pond conditions and other sampling data.

Parameter	Sample A	Sample B
Sampling date	28 August 1981	11 November 1981
Number of fish sampled	60	60
Mean weight (g) of fish ± standard deviation	250.03 ± 31.56	220.07 ± 37.79
Pond conditions		
Pond temperature	35.5°C	34.5°C
pH	8.3	8.4
Salinity[a]	59.86 ppt	13.40 ppt
Dissolved oxygen[b]	8.15 ppm O_2	10.10 ppm O_2
Nitrite-nitrogen	0	0.01 ppm N
Ammonia-nitrogen	0.10 ppm N	0.58 ppm N
Phosphate-phosphorus	0.17 ppm P	0.08 ppm P
Predominant natural food	Unicellular algal complex	Filamentous algae

[a] By titration method.
[b] By modified Winkler method (APHA 1976). Samples of pond water were not fixed.

Lipid Classes

Table 4 shows the results of fractionation of the total lipids by column chromatography using silicic acid. The total lipids were fractionated into three major classes: neutral lipid, phospholipid, and glycolipid.

The neutral lipid content of the unicellular algal complex was higher than that of the filamentous algae. The neutral lipid content of both the depot fat and liver of sample A milkfish was higher than that of sample B. On the other hand, the phospholipid content of the liver and depot fat of sample B was higher than that of sample A. The glycolipid content of the depot fat and liver from both milkfish samples was comparable. Milkfish liver has higher phospholipid and glycolipid contents than depot fat. The depot fat, however, contains more neutral lipids than the liver.

Neutral Lipids

Table 5 shows the results of fractionation of the neutral lipids into their various components by column chromatography using florisil. For milkfish tissues and natural food, the major neutral lipid components were the triglycerides, followed by cholesterol esters. The triglyceride content of the unicellular algal complex is higher than that of the filamentous algae. This trend is reflected in the higher content of triglycerides in both the liver and depot fat of sample A.

Table 2. Typical nutrient composition of natural algal food bases in milkfish aquaculture.

Nutrient component	Percentage dry-weight basis[a]	
	Sample A	Sample B
Crude protein	12.22	12.81
Crude fat	1.98	1.53
Crude fibre	7.46	10.44
Ash	38.83	33.31
Nitrogen-free extract	39.51	41.91

[a] The water content ranged from 78 to 82%.

Table 3. Percentage total lipid content and iodine number of natural food and milkfish tissues.

Sample	Percentage total lipid (wet-weight basis)	Iodine number
Natural food		
Unicellular algal complex	0.92	109.07
Filamentous algae	0.65	94.24
Milkfish depot fat		
Sample A	7.63 ± 1.72	138.13
Sample B	7.89 ± 1.92	136.91
Milkfish liver		
Sample A	2.91 ± 0.56	141.09
Sample B	2.66 ± 0.70	140.72

Fatty-Acid Composition

The fatty-acid composition of the total lipids from natural foods and milkfish tissues was analyzed by gas–liquid chromatography (Table 6). The natural food of the fish contained more saturated than unsaturated fatty acids. A similar trend was observed in the depot fat of both samples A and B. The milkfish liver, however, contained more unsaturated than saturated fatty acids. This trend is clearly reflected in the ratio of saturated to unsaturated fatty acids as well as in the total percentage of saturated and unsaturated fatty acids for the liver tissues analyzed.

In general, the fatty-acid pattern of the depot fat reflected that of the dietary fatty-acid composition. The fatty-acid composition of the liver, however, reflected both dietary patterns and metabolic transformations. Thus, the liver contains several long-chain polyunsaturated fatty acids that were not detected in the milkfish depot fat nor in the natural foods.

Discussion

Milkfish grown by traditional aquaculture feed on natural food that has relatively low lipid content. However, the fish actively metabolize and accumulate lipids from dietary lipids and other nutrients. The major depot fat was found in the muscle of the belly section. Similar to many other fish, milkfish depot fat has a higher lipid content than the liver. However, in other teleosts, such as cod, haddock and hake, practically all of the lipids are localized in their fairly large livers (Brody 1965). The neutral lipid fraction was the most predominant lipid class in milkfish tissues. This was

Table 4. Composition of lipid classes of milkfish tissues and its natural food.

Lipid classes	Natural food		Milkfish liver		Milkfish depot fat	
	A	B	A	B	A	B
Neutral lipid	50.87	46.20	66.87	58.78	78.98	74.35
Phospholipid	38.92	38.35	27.92	35.54	18.82	23.09
Glycolipid	10.18	15.35	5.21	5.66	2.19	2.56

Notes: Lipid classes were resolved by column chromatography as described in the materials and methods section. Classes are expressed as weight percentage of the total lipid.

Table 5. Percentage composition of the neutral lipid fraction of milkfish tissues and its natural food.

Neutral lipid classes	Natural food		Milkfish liver		Milkfish depot fat	
	A	B	A	B	A	B
Hydrocarbons	0.76	1.26	0.40	0.90	0.90	0.80
Cholesterol esters	11.96	16.78	8.50	11.24	12.36	13.86
Triglycerides	73.56	68.30	82.60	71.70	76.24	73.36
Cholesterol	3.60	1.86	1.80	2.70	2.38	2.12
Diglycerides	3.76	3.20	2.80	3.86	2.64	2.80
Monoglycerides	2.78	2.90	1.04	3.76	2.14	2.58
Free fatty acids	3.52	5.38	2.78	5.74	3.22	4.38

Notes: Neutral lipid fractions were resolved by column chromatography as described in the materials and methods section. Fractions are expressed as weight percentage of total neutral lipid.

Table 6. Percentage composition of the major acids in the lipids of milkfish tissues and its natural food.

Fatty acids	Natural food		Milkfish liver		Milkfish depot fat	
	A	B	A	B	A	B
10:0	2.00	0.25	—	—	—	—
12:0	1.14	0.94	1.09	1.14	0.56	0.86
14:0	10.36	9.04	2.07	2.19	4.15	3.30
15:0	2.65	2.83	1.92	1.53	7.22	5.78
16:0	20.72	26.69	14.18	23.59	22.86	31.18
16:1ω7	9.43	10.22	6.06	5.53	16.33	14.67
16:2ω4	6.26	5.86	—	—	1.06	1.65
17:0	6.50	6.41	1.14	1.72	3.59	3.67
17:1ω9	1.90	0.50	2.22	3.37	3.53	3.67
18:0	15.71	17.42	5.05	6.66	11.91	10.27
18:1ω9	15.36	16.56	9.17	20.30	21.83	17.61
18:2ω6	1.90	2.09	2.95	6.66	2.51	2.20
18:3ω3	4.06	0.31	3.45	3.21	1.44	1.10
20:1ω9	1.14	—	3.35	3.09	—	—
20:2ω6	—	—	6.77	1.11	—	—
20:3ω6	0.87	—	2.95	1.29	1.23	—
20:4ω6	—	0.10	4.04	1.74	—	0.43
20:4ω3	—	—	—	—	0.54	—
20:5ω3	—	—	19.60	4.64	—	—
22:1ω9	—	—	2.66	0.18	—	—
22:4ω6	—	—	7.84	8.72	—	0.24
22:5ω6	—	0.75	0.04	0.26	1.23	3.36
22:5ω3	—	—	3.45	3.06	—	—
Total ω3 FAs	4.06	0.31	26.50	10.91	1.98	1.10
Total ω6 FAs	2.77	2.94	24.59	19.78	4.97	6.23
Ratio ω3/ω6	1.47	0.10	1.08	0.55	0.40	0.18
Total saturated FAs	59.08	63.58	25.45	36.83	50.29	55.06
Total unsaturated FAs	40.92	36.39	74.55	63.16	49.70	44.93
Ratio saturated/ unsaturated FAs	1.44	1.74	0.34	0.58	1.01	1.22
Total monoenoic acids	27.83	27.28	23.46	32.47	41.69	35.95
Total polyunsaturated fatty acids	13.09	9.11	51.09	30.69	8.01	8.98

followed by phospholipids and then glycolipids. Earlier studies indicate that neutral lipids are the major constituent of fish lipids and that phospholipids and glycolipids are present in lesser amounts (Kanazawa et al. 1979). The neutral lipids of milkfish consist largely of triglycerides. The same observation was reported for a number of other fish by earlier investigators (Gruger et al. 1964; Lovern 1964; Cowey and Sargent 1979).

The fatty-acid pattern of milkfish depot fat reflected the dietary pattern. The depot fat of the fish contained more saturated than unsaturated fatty acids, a trend also observed in the natural food of the fish. The ratio of the total saturated to total unsaturated fatty acids, as well as the total percentage of polyunsaturated fatty acids (PUFAs), is comparable for both the natural food and depot fat. The results suggest that, in milkfish, dietary fatty acids are deposited essentially unchanged in their adipose tissues.

Previous workers have shown that the fatty-acid composition of depot lipids in fish is greatly influenced by diet. Freshwater eels (*Anguilla vulgaris*) fed exclusively with herring meal containing 20% of a marine type of lipid deposited this dietary component in a virtually unmodified pattern (Lovern 1938). The fatty-acid pattern of channel catfish (*Ictalurus punctatus*) fed three types of dietary lipids (beef tallow, safflower oil, and menhaden oil) was found to be similar to the dietary fatty-acid composition (Stickney and Andrews 1971). The statistical analysis of fatty-acid data on channel catfish grown on six experimental diets containing various sources and amounts of lipids showed that the fatty-acid composition of the fish was strongly influenced by the diet (Worthington and Lowell 1973).

The fatty-acid pattern of the milkfish liver tended to reflect not only the pattern of the dietary lipids but also fatty acids obtained through metabolic transformations of dietary lipids and other nonlipid nutrient components, such as carbohydrates and proteins. The fatty acids from the liver are much more unsaturated than the fatty acids from the depot fat and natural food. The increased unsaturation in the fatty acids from the liver is due to the presence of increased levels of long-chain PUFAs. Significant quantities of 20:2ω6, 20:3ω6, 20:4ω6, 20:5ω3, 22:4ω6, 22:5ω6, and 22:5ω3 fatty acids were found in the liver of both fish samples but not in the milkfish depot fat nor in the natural food. The presence of long-chain PUFAs in the liver, despite their absence in the natural food, suggests that chain elongation and desaturation have occurred and that the site of such metabolic transformations in milkfish is the liver.

The conversion of dietary fatty acids to long-chain PUFAs has been found to occur in fish (Mead et al. 1960; Kayama et al. 1963). Tracer studies on several species of fish, including rainbow trout, ayu, eel, red sea bream, rockfish, and globefish, also showed that by chain elongation and desaturation these fish are capable of converting exogenous 18:3ω3 to 18:4ω3, 20:3ω3, 20:4ω3, 20:5ω3, and 20:6ω3 (Kanazawa et al. 1979).

The mechanism for the synthesis and interconversion of fatty acids in fish is now well established and has shown that fish can synthesize de novo from acetate, fatty acids of the ω5, ω7, ω9, and ω11 series. Fish are unable, however, to synthesize fatty acids of the ω3 and ω6 series unless a precursor with this ω structure is present in the diet (Mead and Kayama 1967; Sinnhuber 1969). This limitation in biosynthetic capability accounts for the essentiality of ω3 and ω6 in most fish (Cowey and Sargent 1979; Castell 1979). The growth-enhancing effect of highly unsaturated ω3 fatty acid, particularly for marine fish, was demonstrated by Takeuchi and Watanabe

(1976, 1977).

The ability of milkfish to grow in natural food bases with relatively low lipid content reflects their capacity to actively metabolize and transform not only dietary fatty acids but also nonlipid nutrient components, such as proteins and carbohydrates.

Acknowledgments

This study was supported by a research grant from the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC). Supplemental support was provided by the International Development Research Centre (IDRC), Canada, and by the Philippine Council for Agricultural Resources Research and Development.

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